



HAL
open science

Iron complexation by phenolic ligands in seawater

Aridane G. Gonzalez, M. Isabel Cadena-Aizaga, Géraldine Sarthou, Melchor Gonzalez-Davila, J.M. Santana-Casiano

► **To cite this version:**

Aridane G. Gonzalez, M. Isabel Cadena-Aizaga, Géraldine Sarthou, Melchor Gonzalez-Davila, J.M. Santana-Casiano. Iron complexation by phenolic ligands in seawater. *Chemical Geology*, 2019, Cycles of trace elements and isotopes in the ocean – GEOTRACES and beyond, 511, pp.380-388. <10.1016/j.chemgeo.2018.10.017>. <hal-02322486>

HAL Id: hal-02322486

<https://hal.science/hal-02322486v1>

Submitted on 2 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

1 **Iron complexation by phenolic ligands in seawater**

2 **Aridane G. González^{1,2*}, M. Isabel Cadena-Aizaga², Geraldine Sarthou¹, Melchor**
3 **González-Dávila², J. M. Santana-Casiano²**

4

5 ¹CNRS, Université de Brest, IRD, Ifremer, UMR 6539 LEMAR, IUEM; Technopôle
6 Brest Iroise, Place Nicolas Copernic, F-29280 Plouzané, France

7 ²Instituto de Oceanografía y Cambio Global, IOCAG. Universidad de Las Palmas de Gran
8 Canaria, ULPGC, Parque Científico Tecnológico de Taliarte, 35214, Telde. Spain.

9 *Corresponding author: aridane.gonzalez@ulpgc.es. Phone: +34 928 454 436

10 **Abstract**

11 Iron is an essential micronutrient for phytoplankton and can limit primary production in
12 the ocean. Fe chemistry is highly controlled by its interaction with organic complexes (>
13 99%). It is still unknown which organic compounds produced by cells have the ability to
14 bind Fe. Within the pool of organic ligands, polyphenols are known to be exuded by
15 marine diatoms and, in this study, the role of three polyphenols ((±) – catechin, sinapic
16 acid and gallic acid) was studied in terms of dissolved Fe complexation via kinetic and
17 titration approaches, and also their role as a source of Fe(II) in seawater. The results
18 demonstrated that these three polyphenols are weak L₂-type Fe-binding ligands according
19 to the conditional stability constant, computed by using the kinetic approach ($\log K'_{FeL} =$
20 $8.86 - 9.2$), where the formation rate constant (k_f) was $3.1 \cdot 10^5 - 4.2 \cdot 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and the
21 dissociation rate constant (k_d) was $2.43 \cdot 10^{-4} - 4.4 \cdot 10^{-4} \text{ s}^{-1}$. The conditional stability was
22 also computed from the titration approach with $\log K'_{FeL}$ from 8.6 to 9.5. These studied
23 ligands also regenerated Fe(II) in seawater from 0.05% to 11.92%. The results obtained
24 in this study suggest that polyphenols increase the persistence of dissolved Fe and should
25 be considered as an important Fe-binding ligands in seawater to better understand the
26 global biogeochemical cycles.

27

28 **Keywords:** Fe-binding ligands, complexation, polyphenols, Fe(III), Fe(II)

29 INTRODUCTION

30 Iron is involved in a variety of metabolic processes and is thus an essential micronutrient
31 for marine microorganisms (Morel and Price, 2003). The low solubility of Fe in seawater
32 (Liu and Millero, 2002) decreases the dissolved concentration of this metal, limiting the
33 growth of phytoplankton in some areas of the ocean, in particular in high nutrient low
34 chlorophyll oceanic regions (Martin et al., 1991). In seawater, the speciation of Fe is
35 highly dominated by the complexation with organic complexes (> 99%), affecting its
36 reactivity (Gledhill and van den Berg, 1994; Rue and Bruland, 1995) and increasing its
37 solubility (Liu and Millero, 2002; Rue and Bruland, 1995), but most of the ligands are
38 unknown. In general, the ligands are studied as natural ligands, a pool of organic ligands
39 able to complex Fe in natural waters, participating in the Fe-uptake system of
40 microorganisms (Shaked and Liss, 2012) by different processes. Adsorption, reduction
41 of Fe(III) to Fe(II) in solution or in the cell wall and complexation of Fe in solution (Morel
42 et al., 2008), and the production of organic ligands by microorganisms can make
43 dissolved Fe (dFe) more bioavailable (Granger and Price, 1999; Hutchins et al., 1999;
44 Maldonado and Price, 1999), although these mechanisms depends on the microorganisms
45 and their environments (Shaked and Liss, 2012).

46 Fe-binding ligands are produced by cells; rupture of cells after grazing (Chase and Price,
47 1997; Hutchins et al., 1995; Hutchins and Bruland, 1994; Sato et al., 2007), viral lysis
48 (Glober et al., 1997; Poorvin et al., 2011), transformation of organic materials (Boyd and
49 Ellwood, 2010; Gerringa et al., 2006; Gledhill and Buck, 2012) and organic exudates
50 from phytoplankton (González-Dávila et al., 1995; Rico et al., 2013; Santana-Casiano et
51 al., 2014; Vasconcelos et al., 2002). In the ocean, the bulk of Fe-binding ligands can be
52 ranked, according to the conditional stability constant (K'_{FeL}), as strong ligands (L_1 - log
53 $K'_{FeL} > 11$) and weak ligands ($L_2 < 11$) (Gledhill and Buck, 2012), where the ligand

54 concentration and the conditional stability constant of the formed complex is measured
55 by competitive ligand exchange adsorptive cathodic stripping voltammetry (CLE-
56 ACSV). However, this technique does not give any information about the molecular
57 structure of organic ligands (Luther et al., 2001). In this sense, the identification of
58 organic functional groups, which form part of the ligands in seawater or cultures, will be
59 relevant in terms of trace metal chemistry.

60 There is a necessity of increase our knowledge about organic functional groups and
61 identify them within the bulk of organic ligands in natural waters. In this sense,
62 polyphenols have been measured in the exudates of two marine microalgae, the marine
63 diatom *Phaeodactylum tricornutum* and the green algae *Dunaliella tertiolecta* (López et
64 al., 2015; Rico et al., 2013; Santana-Casiano et al., 2014), as well as the effect of high
65 metal exposition on the concentration of polyphenols excreted by the cells. The authors
66 reported that the type of phenolic compounds and their concentrations are directly related
67 to the metal concentrations in solution as a mechanism to keep Fe in solution or decrease
68 the free copper concentration due to its toxicity. In addition, polyphenols such as catechol,
69 (\pm) – catechin and sinapic acid can reduce Fe(III) to Fe(II) in seawater (Santana-Casiano
70 et al., 2014).

71 Polyphenols, as other ligands produced by microorganisms (amino acids or
72 polysaccharides), can complex Fe due to the hydroxyl and carboxyl groups on their
73 molecular structures (Andjelković et al., 2006; Brown et al., 1998; Lodovici et al., 2001;
74 Mira et al., 2002; Re et al., 1999; Sroka and Cisowski, 2003), but these Fe-binding
75 complexes have never been studied in seawater in terms of Fe complexation. However,
76 polyphenols have been described as strong dFe-binding ligands in acidic solutions (pH <
77 3; Hynes and O’Coinceanainn (2001, 2004)).

78 The goal of the current investigation is to measure the formation (k_f) and dissociation (k_d)
79 rate constants and the conditional stability constants (K'_{FeL} and K'_{Fe3+L}) for the Fe-
80 complexes with (\pm) – catechin (5,7,3',4'-tetrahydroxyflavan-3-ol), sinapic acid (3,5-
81 dimethoxy-4-hydroxycinnamic acid) and gallic acid (3,4,5-trihydroxybenzoic acid) in
82 seawater. These three compounds have been selected among a variety of polyphenols due
83 to their presence in phytoplankton exudates (López et al., 2015; Rico et al., 2013;
84 Santana-Casiano et al., 2014). These authors reported how these three organic compounds
85 showed a higher concentration in seawater enriched with phytoplankton exudates than in
86 natural seawater. These three compounds allow us to understand the role of three different
87 molecular structures on the chemistry of Fe. In addition, Fe redox experiments were
88 performed in seawater in order to determine the capability of these three model organic
89 ligands to reduce Fe(III) to Fe(II) in seawater. These results will increase our knowledge
90 about Fe biogeochemical cycle and the role of ligands in the chemistry of Fe in the ocean.

91

92 MATERIAL AND METHODS

93 Chemicals

94 Fe was determined by differential pulse cathodic stripping voltammetry (DP-CSV; Croot
95 and Johansson (2000)). A 0.01 M solution of TAC (2-2(2-thiazolylazo)-*p*-cresol) (Sigma-
96 Aldrich) was prepared in HPLC grade methanol (Sigma-Aldrich) once every two weeks
97 and kept in the fridge when it was not in use (darkness and 4°C). An EPPS buffer solution
98 (N-(2-hydroxyethyl)piperazine-N';2-propanesulfonic acid; SigmaUltra) was prepared at
99 1.0 M in 1.0 M NH₄OH (ultrapure, VWR) at pH 8.05. The buffer solution was cleaned
100 three times through an 8HQ (8-Hydroxyquinoline) resin column. Fe(III) stock solutions
101 (1.0 μM and 100 nM) were prepared weekly from a Fe standard solution for atomic

102 absorption spectroscopy (VWR) and acidified at pH 2 with HCl (ultrapure, VWR). The
103 stock solution of Fe(II) ($4 \cdot 10^{-4}$ M) was daily prepared using ammonium Fe(II) sulfate
104 hexahydrate (Sigma), acidified at a pH of 2 with Suprapur HCl (Sigma).

105 The selected organic ligands, (\pm) – catechin, sinapic acid and gallic acid (Sigma-Aldrich)
106 were prepared weekly in HPLC grade methanol at 10^{-3} M and the second stock solutions
107 were prepared, for each experiment, in MQ water (18 M Ω , Millipore) at 10^{-6} M. The
108 molecules of these three selected organic ligands are presented in Figure 1.

109 The reagents were always prepared in 20 mL Teflon (Savilex) vials. These bottles were
110 washed 5 times with MQ water and 2% HCl (suprapure, VWR). The Teflon vials were
111 rinsed 5 times with MQ water prior to use.

112 The seawater used in the current experiments was collected during the oceanographic
113 cruise “GEOVIDE” (station 77, 53.0 °N, -51.1 °E, 40 m) in the North Atlantic Ocean and
114 was filtered on board through 0.2 μ m-pore size cartridge filters. The GEOVIDE seawater
115 (GSW) was kept in the clean laboratory (Class 100) facilities (LEMAR-IUEM) with acid-
116 clean carboy (25L) in the dark, at room temperature, until it was used.

117

118 **Measurements of labile and dissolved Fe**

119 Dissolved Fe (dFe), labile Fe (Fe $'$; all the inorganic species predominantly in the form
120 Fe(OH) $_3$), and dFe-binding ligands (L_{Fe}) concentrations were determined by DP-CSV
121 (Croot and Johansson, 2000) using a μ Autolab voltameter (Metrohm), with a static
122 mercury drop electrode (Metrohm Model VA663), a double-junction Ag/saturated AgCl
123 reference electrode with a salt bridge filled with 3 M KCl, and a glassy carbon rod as a
124 counter electrode. The samples were always processed in a Class 100 clean laboratory at
125 room temperature.

126 Labile Fe was measured in 10 mL seawater samples by adding 100 μ L of EPPS (final
127 concentration 10 mM EPPS buffered to pH 8.05) and 10 μ L of 0.01 M TAC (final
128 concentration 10 μ M). Samples were purged for 180 sec with dry nitrogen gas. A new Hg
129 drop was formed at the end of the purging time. The Teflon vials were conditioned 5
130 times with GSW. The labile Fe concentrations were determined using the method of
131 standard additions. TAC was added at the beginning of the purging time, then the contact
132 time was 180 sec.

133 Dissolved Fe concentrations were measured following the same method, but the samples
134 were previously UV-irradiated during 4 hours in quartz tubes. These tubes were soaked
135 for one day in 10% HCl (suprapure, VWR) and washed with MQ water 5 times prior to
136 use. They were also rinsed one more time with GSW. The Teflon vials were conditioned
137 5 times with GSW. The dFe concentration was determined using the method of standard
138 additions. TAC was also added at the beginning of the purging time, then the contact time
139 was 180 sec.

140 The voltammetric analysis was always the same for all the analysis. The deposition
141 potential of -0.40 V was applied for 180 sec. The sample was stirred during the deposition
142 time. At the end of the deposition time, the scan as a DP-CSV was applied with a
143 modulation time of 0.01 sec, interval time 0.1 sec, initial potential -0.4 V, final potential
144 -0.9 V, step potential 2.55 mV and modulation amplitude 49.95 mV.

145

146 **Dissolved Fe organic speciation measured using the kinetic method**

147 The kinetic of formation was prepared with 10 mL of UV-irradiated GSW (4 hours), 100
148 μ L of EPPS (1 M) buffered to pH 8.05, 10 nM of dFe, and 5 nM of the studied ligands in
149 at least 14 Teflon vials. These experiments were performed with an excess of Fe

150 compared to ligand in order to have enough dFe to saturate the binding sites in the
151 polyphenols and to achieve the maximum complexing capacity. The initial time (t_0)
152 corresponds to the addition of the studied ligand. The addition of TAC (10 μ L of 0.01 M)
153 was performed during the purge, 20 sec before starting the deposition time. This time was
154 used to purge the sample after the addition of TAC. The Fe measured in the kinetic of
155 formation experiments has to be considered as labile Fe (Gerringa et al., 2007), because
156 there is no equilibration time between TAC and the studied ligands due to the short
157 contact time between FeL and TAC. In irradiated seawater samples, this time was found
158 to be enough to measure the labile Fe in solution.

159 The kinetic of dissociation of dFe-ligand complexes was prepared in at least 14 Teflon
160 vials with 10 mL of UV-irradiated GSW (4 hours), 100 μ L of EPPS (1 M) buffered to pH
161 8.05, 10 nM of Fe and 5 nM of the studied ligand. The times of equilibration for each
162 ligand were defined, according to the formation results, when the plateau was reached (4
163 hrs for (\pm) – catechin, 2 hrs for sinapic acid, and 15 hrs for gallic acid). Then 10 μ L of
164 TAC (0.01 M) was added into the samples, corresponding with the initial time of
165 dissociation (t_0). In this case, as TAC and the studied ligand can compete during the
166 dissociation, the measured dFe is here called ‘TAC labile Fe’ (Gerringa et al., 2007). The
167 addition of TAC will form an electroactive complex which can be detected in the mercury
168 drop.

169 All the experiments were carried out in triplicate and the Teflon vials were conditioned 5
170 times with GSW.

171

172 **Dissolved Fe organic speciation measured by DP-CSV**

173 In a series of 14 Teflon bottles an aliquot of 10mL of natural seawater, 100 μ L of EPPS
174 (1 M) buffered to pH 8.05, and different concentrations of dFe (from 0 to 15 nM) were
175 pipetted into the bottles. For GSW titrations, the solution was left to equilibrate for one
176 hour. Then, 10 μ L of TAC (0.01 M) were added and left to equilibrate overnight (Croot
177 and Johansson, 2000). The samples were measured in a Teflon cell. First, two GSW sub-
178 samples were systematically analysed according to the recommendation of the
179 GEOTRACES program, where two +0-dFe additions in the titration with at least eight
180 dFe additions, for a total of 10 or more titration points will provide a better data
181 interpretation (Garnier et al.,2004; Gledhill and Buck, 2012; Sander et al., 2011).

182 The dFe-binding capacities of the studied organic ligands, (\pm) – catechin, sinapic acid and
183 gallic acid, were measured following the same method as indicated in the titration
184 approach. A solution containing 10 mL of UV-irradiated GSW (UV-treatment for 4
185 hours), 100 μ L of EPPS (1 M) buffered to pH 8.05, 0 to 15 nM of Fe(III) and 2 – 11.8
186 nM of the studied ligand was prepared. These solutions were equilibrated for the same
187 time as above and, 20 sec before the deposition time, 10 μ L of TAC (0.01 M) were
188 pipetted into the Teflon cell for measurement. This short time was selected in order to
189 avoid equilibrium between TAC and FeL, and because the interest of this work was to
190 measure the equilibrium between Fe and polyphenols.

191 The titration data were processed using the ProMCC program (Omanović et al., 2014) in
192 order to compute the concentrations of ligands and the conditional stability constants.

193

194 **Fe(III) reduction experiments**

195 The Fe(III) reduction experiments were carried out according to Santana-Casiano et al.
196 (2010, 2014). Briefly, a 100 nM concentration of Fe(II) was used in 100 ml of seawater

197 in a 200 ml thermostatted vessel controlled to 25 ± 0.02 °C and pH 8.0 ± 0.01 . The pH
198 of the solution was determined on the free scale, pH_F (Millero, 1986). The pH was
199 adjusted to the desired value with additions of small amounts of 1 M HCl, automatically
200 controlled by a 719 titrinoTM (Methrom) to keep it constant. This Fe concentration was
201 used to reproduce the same experiments as carried out by Santana-Casiano et al. (2010,
202 2014) and to ensure that the preparation of the polyphenols stock solutions in methanol
203 and MQ did not have any effect on the Fe(II) reduction process and be able to compare
204 the current study with the literature. The solution was initially aerated with air for two
205 hours under oxygen saturation conditions in order to fully oxidize the added Fe(II)
206 solution. After that time, 10^{-4} M ferrozine (FZ) was added. Fe(II) was not detected in the
207 absence of the phenolic compounds. The addition of 100 nM of the polyphenol
208 corresponds to zero time of reaction. The Fe(II)-FZ complex was recorded over time at
209 562 nm in a 5-m-long waveguide capillary flow cell (LWCFC) from World Precision
210 Instruments connected to the UV detector S4000 (Ocean OpticsTM). This technique
211 allows to measure both Fe(II) and the organic compounds. Every acquisition time, the
212 absorbance spectrum was analysed to correct the baseline (Santana-Casiano et al., 2010).
213 These experiments will enable us to detect if Fe(II) is formed during the equilibration
214 time between dFe and the studied polyphenols.

215

216 **RESULTS**

217 **Dissolved Fe concentration in the seawater**

218 The dFe in the natural seawater used in the current investigation was 0.36 ± 0.06 nM (n
219 = 8) and the Fe' was 0.020 ± 0.004 nM (n = 8). Then, the FeL in the natural seawater
220 sample was ~95%. Figure 2 shows one of the titrations carried out by additions of Fe (0
221 – 15 nM) to the GSW and the UV-irradiated GSW. These results show that the natural

222 ligand concentration calculated using the ProMCC code was 3.1 ± 0.7 nM ($n = 10$). This
223 seawater contained 8 times more ligands than dFe. The conditional stability constant was
224 $\log K'_{\text{Fe}^{\text{L}}}=11.5 \pm 0.4$ in respect to Fe' and $\log K'_{\text{Fe}^{\text{3+L}}} = 21.5$. Therefore, the ligands in
225 GSW are ranked as L₂-type ligands (Gledhill and Buck, 2012). No significant differences
226 were observed between linear (van den Berg and Kramer, 1979) and non-linear treatment
227 (Gerringa et al., 2014; Pižeta et al., 2015). The standard error from the ProMCC for each
228 analysis in terms of $K'_{\text{Fe}^{\text{L}}}$ and ligand concentration was always at least one order of
229 magnitude lower than the same parameter.

230

231 **Dissolved Fe speciation from the kinetic approach**

232 In order to study the Fe-binding capacity of polyphenols in terms of kinetic experiments,
233 the GSW was UV-irradiated during 4 hours. This was long enough to decompose all the
234 organic ligands, as demonstrated by the linear dependence of the peak high (nA) and the
235 Fe concentrations (Figure 2). The concentration of dFe did not exceed the solubility of
236 Fe in our seawater samples because the standard additions were linear in the studied range
237 (15 nM of dFe). However, the linear standard additions can also occur when formation
238 kinetics are quick and TAC dissolves amorphous Fe oxides rapidly. The height peak (dFe
239 concentration) after 10 nM of dFe addition was followed in UV-irradiated GSW by more
240 than 24 h and it was stable. These experiments were also done by triplicate in at least 14
241 Telfon vials.

242 The kinetic approach was performed according with Witter and Luther (1998) and Wu
243 and Luther (1995). The $K'_{\text{Fe}^{\text{L}}}$ can be estimated from k_f and k_d by following Equation 1
244 where k_f and k_d where computed from the experimental results showed in Figure 3.

245

246
$$K'_{Fe'L} = k_f/k_d \tag{1}$$

247

248 In addition, the conditional stability constant with respect to Fe^{3+} ($K'_{Fe^{3+}L}$) can be
 249 calculated by assuming that the second-order rate constant is $k_{f,Fe^{3+}L} = 3.02 \cdot 10^{11} M^{-1} s^{-1}$,
 250 previously estimated from encounter theory (Luther and Wu, 1997).

251

252
$$K'_{Fe^{3+}L} = \alpha_{Fe'} K'_{Fe'L} \tag{2}$$

253

254 where $[Fe']$ is labile Fe as was described above and Fe^{3+} is the free inorganic Fe
 255 (Stockdale et al., 2016), $\alpha_{Fe'} = 10^{10}$ (Hudson et al., 1992; Sunda and Huntsman, 2003), is
 256 commonly used for pH 8 seawater. Then, the dissociation rate constant $k_{d, Fe^{3+}L}$ can be
 257 computed (Equation 3).

258

259
$$k_{d,Fe^{3+}L} = \frac{k_{f,Fe^{3+}L}}{K'_{Fe^{3+}L}} \tag{3}$$

260

261 The half-life time ($t_{1/2}$), for $Fe'L$ and $Fe^{3+}L$, has also been computed according to the
 262 pseudo-first kinetic equation, which can be expressed as: $t_{1/2} = \ln 2/k_d$.

263 In addition, the presence of organic ligands enhances Fe solubility by favoring the
 264 formation of small Fe soluble species (Kuma et al., 1996).

265 The results for the kinetic of formation and dissociation experiments are shown in Figure
 266 3 and Table 1. They showed that the dFe – phenolic complexes are rapidly formed with
 267 $\log k_f$ between 5.50 – 5.62 (k_f in $M^{-1} s^{-1}$, Table 1). On the other hand, the dissociation rate
 268 constants were also high; $\log k_d$ -3.36 – 3.61 (k_d in s^{-1} , Table 1) for these polyphenol

269 groups. Then, the $\log K'_{Fe'L}$ determined from the kinetic experiments were equal to $9.2 \pm$
270 0.1 ((\pm) – catechin), 8.86 ± 0.04 (sinapic acid) and 9.01 ± 0.02 (gallic acid)), relative to
271 the Fe' species. These Fe-organic complexes can be defined as weak ligands (L_2 -type
272 ligands; Gledhill and Buck (2012)). According to the dissociation rate constants, the
273 presence of polyphenols will keep dFe in solution from 11.3 min to 20.6 min, in respect
274 to the complex of Fe' with sinapic acid or (\pm) – catechin, respectively. This half-life time
275 increased to the order of years ($0.32 - 0.54$) in terms of Fe^{3+} (Table 1). This difference is
276 due to the calculation of half-life time for $Fe^{3+}L$ complexes assumes an upper diffusion
277 control limit to formation of the complex rather than the activation control for $Fe'L$
278 (Witter et al., 2000).

279

280 **Conditional stability constants from CLE-CSV**

281 The total amounts of organic ligands in seawater can be computed using Equation (4).

282

$$283 \quad [L_T] = [L'] + [FeL] \quad (4)$$

284

285 where $[L_T]$ is the total concentration of ligands, $[L']$ includes both free ligands and ligands
286 complexed with other major cations in seawater (as Ca^{2+} , Mg^{2+} and H^+ ; Rue and Bruland
287 (1995)), $[FeL]$ the concentration of Fe-binding ligands. In the same sense, the
288 concentration of Fe in solutions can be considered as Equation 5.

289

$$290 \quad [Fe]_T = [Fe'] + [FeL] \quad (5)$$

291

292 where $[Fe]_T$ represents the total dissolved Fe in seawater, $[Fe']$ represents the
293 concentration of all the inorganic species (predominantly in the form $Fe(OH)_3$ (Stockdale
294 et al., 2016). The equilibrium system between Fe and the organic ligand is:

295



297

298 where the conditional stability constant with respect to $[Fe']$ is expressed as Equation 7:

299

$$300 \quad K'_{FeL} = \frac{[FeL]}{[Fe'][L']} \quad (7)$$

301

302 The relationship between Fe' and Fe^{3+} can be used as: $\alpha_{Fe'} = [Fe']/[Fe^{3+}]$, then $K'_{Fe'L} =$
303 $\alpha_{Fe'} K'_{Fe^{3+}L}$, where $\alpha_{Fe'} = 10^{10}$ (Hudson et al., 1992; Sunda and Huntsman, 2003) is
304 commonly used for pH 8 seawater and the $K'_{Fe'L}$ can then be calculated from the values
305 of Fe' measured by CSV and the dFe and L concentrations using Equations 4, 5 and 7.

306 The complexation of dFe by the three different polyphenols, (\pm) – catechin, sinapic acid
307 and gallic acid, was studied in UV-irradiated GSW (Table 2). Two different
308 concentrations of the studied Fe-binding ligands were used in order to evaluate if the
309 binding capacity was related to the concentration and to determine if the binding sites
310 were saturated with those Fe levels in every sample. The ligand (\pm) – catechin was able
311 to complex 1.6 ± 0.2 nM and 3.6 ± 0.5 nM of dFe when 5.7 and 11.8 nM of the studied
312 ligand were present in solution, with a $\log K'_{Fe'L}$ between 9.3 – 9.1, respectively. Sinapic
313 acid complexed 3.6 ± 0.8 and 6.5 ± 0.7 nM of dFe when 5.0 and 9.9 nM of the ligand
314 were added, with a $\log K'_{Fe'L} = 8.6$. Finally, when 2.0 nM of gallic acid was added, $5.5 \pm$
315 0.3 nM of dFe was organically complexed and when 5 nM of gallic acid was added to the
316 UV-seawater, 14 ± 2 nM of dFe were found complexed. The amount of Fe complexed or

317 removed by gallic acid was always over double the added ligand concentration. The log
318 K'_{FeL} values were 8.8 and 9.5, respectively.

319 Taking into account the titration approach, the log K'_{FeL} was from 8.6 to 9.5 (Table 2).
320 Accordingly, the studied ligands formed weak organic complexes with dFe in seawater,
321 with a conditional stability constant ≥ 18 with respect to Fe^{3+} . Then, they can be classified
322 as L₂-type organic ligands (Gledhill and Buck, 2012).

323

324 **Fe(III) reduction by polyphenols in seawater**

325 Fe(III) is reduced by (±) – catechin, sinapic acid and gallic acid in UV-irradiated GSW at
326 pH 8.0 and 25°C (Figure 4). The Fe(III) reduction rate constant (pseudo-first order rate
327 constant) was $8.98 \pm 0.02 \cdot 10^{-7} \text{ sec}^{-1}$, $1.45 \pm 0.02 \cdot 10^{-7} \text{ sec}^{-1}$ and $3.90 \pm 0.04 \cdot 10^{-5} \text{ sec}^{-1}$ for
328 (±) – catechin, sinapic acid and gallic acid, respectively. This means that 100 nM of
329 polyphenol are able to reduce from 0.05 nM to 11.92 nM of Fe(III) in one hour of reaction
330 in UV-irradiated GSW. Then, at equimolar concentration (100 nM of dFe and the studied
331 organic ligand), the studied polyphenols were able to reduce Fe(III) to Fe(II) in seawater.

332

333 **DISCUSSION**

334 **Polyphenols as dFe-complexing ligands in seawater**

335 Recent investigations (López et al., 2015; Rico et al., 2013; Santana-Casiano et al., 2010,
336 2014) demonstrated that polyphenols have to be considered as important organic ligands
337 within the Fe biogeochemical cycle due to their ability to reduce Fe(III) to Fe(II) in
338 seawater as well as to be produced in the microalgae exudates as a response to the Fe
339 exposition. According to the molecular structure (Figure 1), (±) – catechin is a flavonoid

340 type compound with a catechol moiety in the B-ring, a resorcinol group in the A-ring and
341 a hydroxyl group at position 3 in the C-ring (Figure 1A). Sinapic acid is a
342 phenylpropanoid compound, with 3,5-dimethoxyl and 4-hydroxyl groups substituting the
343 phenyl group of the cinnamic acid (Figure 1B). Gallic acid is tri-hydroxybenzoic acid
344 (gallyol moiety) (Figure 1C). Such acids are generally forming dimers such as ellagic
345 acid. (\pm) – catechin, sinapic acid and gallic acid contain –OH groups in their molecules
346 which at pH \sim 8 (natural seawater pH) should be deprotonated forming anionic ligands
347 capable of complexing metals, as the organic ligands at the cell surface of microorganisms
348 (González et al., 2014). Accordingly, polyphenols at natural pH complex dFe in seawater,
349 as it was previously reported at acidic pH and low ionic strength solutions (Andjelković
350 et al., 2006; Brown et al., 1998; Hynes and O’Coinceanainn, 2004; Khokhar and Apenten,
351 2003; Mira et al., 2002).

352 In general, the dFe complexation by polyphenols is related to the presence of the *ortho*-
353 di-hydroxy groups, mainly present in molecules bearing catechol or gallyol moieties
354 (Khokhar and Apenten, 2003; Moran et al., 1997) and to the amount of –OH groups in
355 each molecule. In fact, Andjelković et al. (2006) demonstrated that polyphenols bearing
356 gallyol groups form stronger chelates than molecules with catechol moiety in aqueous
357 solutions. The formation of complexes between dFe and the organic ligands proposed in
358 this investigation showed that gallic acid is the most important phenolic compound in
359 terms of dFe-binding capacity, where a stoichiometric ratio 1:1 can be assumed (Table
360 2). The differences in the complexing capacity could not only be understood as a role of
361 the hydroxyl groups in the catechol or gallyol moieties in seawater. The presence of major
362 ions such as Mg^{2+} and Ca^{2+} play a key role in the oxidation process of polyphenols,
363 blocking the reaction to semiquinone and benzoquinone (Santana-Casiano et al., 2010,
364 2014). Then, some of the –OH groups are occupied by Mg^{2+} and Ca^{2+} , decreasing their

365 capacity to complex dFe (Santana-Casiano et al., 2010, 2014). In addition, the ability of
366 gallic acid to form dimers in solution can be involved in the higher dFe-binding capacity
367 compared to that for (±) – catechin and sinapic acid.

368 The kinetics of formation did not reveal a very strong decrease in the labile Fe in the first
369 seconds (Figure 3), as it occurs for stronger ligands, L₁-type ligands, such as
370 protoporphyrin IX (Witter et al., 2000), with a decrease of 2 nM of Fe within the first
371 seconds of the reaction, supporting the experimental results for polyphenols that are
372 ranked as weak ligands. The polyphenols studied in this manuscript progressively
373 decreased the labile Fe over time (Figure 3). Other studied ligands have also been
374 characterized in terms of formation rate constant in seawater (Witter et al., 2000) such as
375 protoporphyrin IX, phaeophytin, apoferritin, phytic acid, alterobactin A and B,
376 ferrichrome and desferrioxamine, with k_f from $0.93 \cdot 10^5$ to $19.6 \cdot 10^5 \text{ M}^{-1} \text{ s}^{-1}$. These authors
377 (Witter et al., 2000), also reported the dissociation rate constant for the same organic
378 binding ligands and ranked from $0.05 \cdot 10^{-6}$ to $15.8 \cdot 10^{-6} \text{ s}^{-1}$. The data collected from the
379 literature for natural waters show a high variability (from $0.31 \cdot 10^{-6}$ to $39 \cdot 10^{-6}$; Luther et
380 al. (1997) and Witter et al. (1998), which proves the huge diversity of ligands in the water.

381 The dissociation rate constant allows to compute the half-life time of Fe in solution, both
382 as Fe' and Fe³⁺ (Table 1). In the presence of polyphenols, Fe' half-life time is 11 – 21 min,
383 which increases to 0.3 – 0.5 years when Fe³⁺ species are considered. It means that Fe
384 persists in solution longer periods when polyphenols are present. These $t_{1/2}$ are highly
385 affected by the presence of organic ligands in the ocean and ranked from minutes to years
386 (Witter et al., 2000).

387 The log K'_{FeL} values estimated from the kinetic experiments for (±) – catechin, sinapic
388 acid and gallic acid are weaker than other model ligands studied by Witter et al. (2000)
389 which log K'_{FeL} values were 10.8 – 13.0, and also with humic substances (log K'_{FeL} =

390 11.1; Laglera et al. (2011)) and fulvic acids ($\log K'_{\text{FeL}} = 10.6-10.9$; Laglera et al. (2011);
391 Laglera and van den Berg (2009), exudates from *Pseudonana antarctica* ($\log K'_{\text{FeL}} =$
392 11.9; Norman et al., 2015) and *Emiliania huxleyi* ($\log K'_{\text{FeL}} = 11.9$; Boye and van den
393 Berg, 2000).

394 According to the recovery of ligands with respect to the initial addition, a tentative
395 stoichiometry can be computed. The (\pm) – catechin and sinapic acid followed the same
396 trend, the concentration of the recovered ligand was always lower than the added ligand,
397 and the recovered ligand concentration in the titration approach (Table 2) is comparable
398 with the FeL formation in the kinetic experiments (Figure 3). However, the addition of a
399 certain concentration of gallic acid always resulted in a higher concentration of recovered
400 ligand or FeL formation in both approaches (Table 2 and Figure 3). The addition of (\pm) –
401 catechin was increased from 5.7 to 11.8 nM (2.07-fold) and the recovered ligands was
402 2.25-fold. Attending to sinapic acid, the added level increased by 2.0 (Table 2) and the
403 recovered ligand increased by 1.8-fold. Then, a tentative stoichiometry of 1:1 ligand-to-
404 metal complexes can be assumed. The gallic acid is the only case where the addition of a
405 certain level resulted in a higher recovered concentration. Then, in this case, the
406 relationship between FeL and added L was ~ 3 (Table 2). In the literature, there are
407 conflicting results for Fe-gallic acid complexes where the stoichiometry can be from 1:1
408 to 1:3 metal-to-ligand complexes (Andjelković et al., 2006; Fazary et al., 2008; Powell
409 and Taylor, 1982; Strlič et al., 2002) as well as 1:1 and 2:1 metal-to-ligand complexes
410 (Hynes and O'Coinceanainn, 2001), demonstrating the complicated nature of these type
411 of ligands in solution, where the binding capacity is function of the pH, the composition
412 of the solution, the reactivity of the gallic acid to reduce Fe(III) to Fe(II) and the formation
413 of other molecules such as quinone, semiquinone or tannic acids (Andjelković et al.,
414 2006; Fazary et al., 2008; Powell and Taylor, 1982; Strlič et al., 2002) which could

415 complex Fe in a higher ratio. In addition, Strlič et al. (2002) reported the role of the
416 Fe:gallic acid complexes in terms of the HO[•] production that also have an impact on Fe
417 redox chemistry. Then, further experiments will be carried out in order to elucidate the
418 Fe:gallic acid stoichiometry in seawater. For this purpose, an evaluation of binding
419 capacity will be studied by using a combination of UV-Vis absorption spectroscopy
420 (Andjelković et al., 2006) and voltammetric technique (this study) as a function of dFe
421 and gallic acid concentration in seawater.

422

423 **Polyphenols as a source of Fe(II) in seawater**

424 The complexation of dFe with polyphenols can be considered as a first step in the process
425 of internalization of Fe by phytoplankton, reducing Fe(III) to Fe(II) both in seawater
426 (Santana-Casiano et al., 2010, 2014) and at cell surfaces (González et al., 2014). The
427 same mechanism was also studied for catechol (Santana-Casiano et al., 2010), (±) –
428 catechin and sinapic acid (Santana-Casiano et al., 2014) as a function of pH. Polyphenols
429 form a complex with Fe(III) and reduce a fraction of Fe(III) to Fe(II), as it has been shown
430 in the current manuscript. The reduction rate constants (in log k') for (±) – catechin and
431 sinapic acid are comparable with those measured by Santana-Casiano et al. (2014), where
432 k' was $7.02 \cdot 10^{-7} \text{ s}^{-1}$ and $2.67 \cdot 10^{-7} \text{ s}^{-1}$, respectively. Gallic acid showed a higher log k' that
433 is in the same range as that for catechol (Santana-Casiano et al., 2010). In addition, the
434 organic exudates excreted by *P. tricornerutum* also retarded the Fe(II) oxidation rate
435 constant in seawater (González et al., 2012; Santana-Casiano et al., 2014). From the
436 current results, we can conclude that organic exudates such as polyphenols increase the
437 persistence of Fe(II) in seawater, either by decreasing their oxidation rate constant to
438 Fe(III) and/or by reducing Fe(III) to Fe(II).

439

440 **Possible discrepancies between kinetic and titration approaches**

441 The experimental methods used in the current manuscript will allow to determine the
442 conditional equilibrium constant for Fe-binding ligands as well as the concentration of
443 these ligands in solution. The calculation of these parameters is highly dependent on the
444 pH and major ions (Laglera et al., 2001).

445 In the current manuscript, the comparison between kinetic and titration approaches are in
446 a good agreement for (\pm) – catechin and sinapic acid, but there are significant differences
447 in the determination of FeL concentration when gallic acid was studied. These differences
448 can be understood as a consequence of the combination of a number of possible factors
449 acting on the experimental approach, such as Fe(II) production during the formation
450 reaction time, the oxidation of gallic acid to semiquinone or quinone groups and the
451 formation of other organic molecules from gallic acid. In the experimental approach, all
452 non-labile Fe is assumed to be complexed by the organic ligand, which can be the case
453 for catechin and sinapic acid, while for gallic acid, other processes could be acting.
454 Therefore, the constant provided in this study should be considered a global complexation
455 constant for any Fe present in the solution. There is an important discrepancy about the
456 Fe:gallic acid stoichiometry in the literature because its reaction mechanism dependent
457 on pH, ionic strength and concentrations of both chemical (Andjelković et al., 2006;
458 Fazary et al., 2008; Hynes and O’Coinceanainn, 2001; Powell and Taylor, 1982; Strlič et
459 al., 2002). Further experiments are necessary to properly define the Fe:gallic acid
460 stoichiometry in seawater by a combination of techniques and physico-chemical
461 conditions.

462 Accordingly, the Fe(II) regeneration during the formation reaction and during the
463 equilibration between dFe and polyphenols can also invoke a slight discrepancies in terms
464 of ligand concentration and conditional stability constant. During the equilibration time
465 between Fe and the studied ligands (> 2 hours), a fraction of Fe(III) can be reduced to
466 Fe(II) and when TAC is added to the titration vials, this fraction of Fe is not reacting with
467 TAC as Fe(III). It was determined that during 1 h of equilibration between dFe and the
468 studied polyphenols, from 0.05% to 11.92% of Fe(II) is formed. Then, the calculation of
469 the conditional stability constant and ligand concentration from the titration can be
470 overestimated.

471 In the case of polyphenols, the interaction with Mg^{2+} and Ca^{2+} affects the oxidation of the
472 semiquinone radical to quinone and it may affect the Fe chemistry, both in terms of
473 complexation and in terms of redox reactions, and it has been demonstrated for catechol,
474 (\pm) – catechin and sinapic acid (Santana-Casiano et al., 2010, 2014). Moreover, during
475 the equilibration time between Fe and the studied ligands, the competition with the MgL
476 and CaL could affect the dFe-ligand complexation (Hering and Morel, 1988; Raspor et
477 al., 1980; Wu and Luther, 1995), which becomes more relevant when weak ligands are in
478 solution. This competition between Fe with Ca and Mg for the binding sites can introduce
479 an interference for the calculation of the ligand concentration as well as of the conditional
480 stability constant.

481 These artefacts are more relevant for weak ligands than for strong ligands. Therefore, the
482 kinetic approach seems more consistent for use in future experiments defining the
483 complexation of dFe and studied ligands in seawater.

484

485 **CONCLUSIONS**

486 Polyphenols as model dFe-binding ligands are excreted by phytoplankton and can play
487 two different roles in terms of the Fe biogeochemical cycle. On one hand, the polyphenols
488 can complex dFe in seawater, and on the other hand, these polyphenols can reduce Fe(III)
489 to Fe(II). Among the pool of binding ligands, (\pm) – catechin, sinapic acid and gallic acid
490 have been thermodynamically characterized. The formation rate constant (k_f) measured
491 for the complex between dFe and (\pm) – catechin, sinapic acid and gallic acid, varied from
492 $3.14 \cdot 10^5$ to $4.17 \cdot 10^5$ ($M^{-1}s^{-1}$). The dissociation rate constant (k_d) varied from $2.43 \cdot 10^{-4}$ to
493 $4.4 \cdot 10^{-4}$ (s^{-1}). The conditional stability constants using the kinetic approach were also
494 calculated ($\log K'_{FeL} = 8.86 - 9.2$). These conditional stability constants were compared
495 with the titration approach, where the $\log K'_{FeL}$ values were from 8.6 to 9.5 for the three
496 studied Fe-binding ligands. These polyphenols are within the L_2 -type ligands. Moreover,
497 Fe(III) is reduced by (\pm) – catechin, sinapic acid and gallic acid in seawater, promoting
498 the formation of Fe(II) from 0.05% to 11.92%. Taking these results into consideration,
499 polyphenols should be considered as an important dFe-binding ligands in seawater in
500 order to properly understand the biogeochemical cycle of Fe and organic ligands.

501

502 **References**

- 503 Andjelković, M., Van Camp, J., De Meulenaer, B., Depaemelaere, G., Socaciu, C.,
504 Verloo, M., Verhe, R., 2006. Iron-chelation properties of phenolic acids bearing
505 catechol and galloyl groups. Food Chem. 98, 23–31.
506 <https://doi.org/10.1016/j.foodchem.2005.05.044>
- 507 Boyd, P.W., Ellwood, M.J., 2010. The biogeochemical cycle of iron in the ocean. Nat.
508 Geosci. 3, 675–682. <https://doi.org/10.1038/ngeo964>
- 509 Boye, M., van den Berg, C.M.G., 2000. Iron availability and the release of iron-

510 complexing ligands by *Emiliana huxleyi*. Mar. Chem. 70, 277–287.
511 [https://doi.org/10.1016/S0304-4203\(00\)00032-3](https://doi.org/10.1016/S0304-4203(00)00032-3)

512 Brown, J.E., Khodr, H., Hider, R.C., Rice-Evans, C.A., 1998. Structural dependence of
513 flavonoid interactions with Cu²⁺ ions: implications for their antioxidant properties.
514 Biochem. J. <https://doi.org/10.1042/bj3301173>

515 Chase, Z., Price, N.M., 1997. Metabolic consequences of iron deficiency in heterotrophic
516 marine protozoa. Limnol. Oceanogr. 42, 1673–1684.
517 <https://doi.org/10.4319/lo.1997.42.8.1673>

518 Croot, P.L., Johansson, M., 2000. Determination of iron speciation by cathodic stripping
519 voltammetry in seawater using the competing ligand 2-(2-thiazolylazo)-p-cresol
520 (TAC). Electroanalysis 12, 565–576.

521 Fazary, A.E., Taha, M., Ju, Y.H. 2008. Iron complexation studies of gallic acid. Journal
522 of Chemical and Engineering Data, 54(1), 35–42.

523 Garnier, C., Pižeta, I., Mounier, S., Benaim, J. Y., & Branica, M. (2004). Influence of the
524 type of titration and of data treatment methods on metal complexing parameters
525 determination of single and multi-ligand systems measured by stripping
526 voltammetry. Anal. Chim. Acta. 505(2), 263–275.

527 Gerringa, L.J.A.A., Veldhuis, M.J.W.W., Timmermans, K.R., Sarthou, G., de Baar,
528 H.J.W.W., 2006. Co-variance of dissolved Fe-binding ligands with phytoplankton
529 characteristics in the Canary Basin. Mar. Chem. 102, 276–290.
530 <https://doi.org/10.1016/j.marchem.2006.05.004>

531 Gerringa, L.J.A., Herman, P.M.J., Poortvliet, T.C.W., 1995. Comparison of the linear van
532 den Berg/Ružić transformation and a non-linear fit of the Langmuir isotherm applied

533 to Cu speciation data in the estuarine environment. *Mar. Chem.* 48, 131–142.
534 [https://doi.org/10.1016/0304-4203\(94\)00041-B](https://doi.org/10.1016/0304-4203(94)00041-B)

535 Gerringa, L. J., Rijkenberg, M. J., Thuróczy, C. E., Maas, L. R., 2014. A critical look at
536 the calculation of the binding characteristics and concentration of iron complexing
537 ligands in seawater with suggested improvements. *Environ. Chem.* , 11(2), 114-136.

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

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

545 Gledhill, M., van den Berg, C.M.G., 1994. Determination of complexation of iron (III)
546 with natural organic complexing ligands in seawater using cathodic stripping
547 voltammetry. *Mar. Chem.* 47, 41–54. [https://doi.org/10.1016/0304-4203\(94\)90012-](https://doi.org/10.1016/0304-4203(94)90012-4)
548 4

549 Glober, C.J., Hutchins, D.A., Fisher, N.S., Coper, E.M., Sañudo-Wilhelmy, S.A., 1997.
550 Release and bioavailability of C, N and Fe following virial lysis of a marine
551 chrysophyte. *Limnol.Oceanogr.* 42, 1492–1504.

552 González-Dávila, M., Santana-Casiano, J.M., Pérez-Peña, J., Millero, F.J., 1995. Binding
553 of Cu(II) to the surface and exudates of the alga *Dunaliella tertiolecta* in seawater.
554 *Environ. Sci. Technol.* 29, 289–301.

555 González, A.G., Pokrovsky, O.S., Jiménez-Villacorta, F., Shirokova, L.S., Santana-

556 Casiano, J.M., González-Dávila, M., Emnova, E.E., 2014. Iron adsorption onto soil
557 and aquatic bacteria: XAS structural study. *Chem. Geol.* 372, 32–45.
558 <https://doi.org/10.1016/j.chemgeo.2014.02.013>

559 González, A.G., Santana-Casiano, J.M., González-Dávila, M., Pérez, N., 2012. Effect of
560 organic exudates of *Phaeodactylum tricornutum* on the Fe(II) oxidation rate
561 constant. *Ciencias Mar.* 38.

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

564 Hering, J.G., Morel, F.M.M., 1988. Kinetics of trace metal complexation: role of alkaline-
565 earth metals. *Environ. Sci. Technol.* 22, 1469–1478.
566 <https://doi.org/10.1021/es00177a014>

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

571 Hutchins, D.A., Bruland, K.W., 1994. Grazer-mediated regeneration and assimilation of
572 Fe, Zn and Mn from planktonic prey. *Mar. Ecol. Prog. Ser.* 110, 259–269.

573 Hutchins, D.A., Wang, W.X., Fisher, N.S., 1995. Copepod grazing and the
574 biogeochemical fate of diatom iron. *Limnol. Oceanogr.* 40(5), 989–994.
575 <https://doi.org/10.4319/lo.1995.40.5.0989>

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

579 Hynes, M., O’Coinceanainn, M., 2001. The kinetics and mechanisms of the reaction of
580 iron (III) with gallic acid, gallic acid methyl ester and catechin. *Journal of Inorganic*
581 *Biochemistry*, 85(2–3), 131–142.

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

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

588 Kuma, K., Nishioka, J., Matsunaga, K., 1996. Controls on iron(III) hydroxide solubility
589 in seawater: The influence of pH and natural organic chelators. *Limnol. Oceanogr.*
590 41, 396–407. <https://doi.org/10.4319/lo.1996.41.3.0396>

591 Laglera-Baquer, L. M., González-Dávila, M., and Santana-Casiano, J. M., 2001.
592 Determination of metallic complexing capacities of the dissolved organic material
593 in seawater. *Scientia Marina*, 65(S1), 33-40.

594 Laglera, L.M., Battaglia, G., van den Berg, C.M.G., 2011. Effect of humic substances on
595 the iron speciation in natural waters by CLE/CSV. *Mar. Chem.* 127, 134–143.
596 <https://doi.org/10.1016/j.marchem.2011.09.003>

597 Laglera, L.M., van den Berg, C.M.G., 2009. Evidence for geochemical control of iron by
598 humic substances in seawater. *Limnol. Oceanogr.* 54, 610–619.
599 <https://doi.org/10.4319/lo.2009.54.2.0610>

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

602 Lodovici, M., Guglielmi, F., Casalini, C., Meoni, M., Cheynier, V., Dolara, P., 2001.
603 Antioxidant and radical scavenging properties in vitro of polyphenolic extracts from
604 red wine. *Eur. J. Nutr.* 40, 74–77.

605 López, A., Rico, M., Santana-Casiano, J.M., González, A.G., González-Dávila, M., 2015.
606 Phenolic profile of *Dunaliella tertiolecta* growing under high levels of copper and
607 iron. *Environ. Sci. Pollut. Res.* 22, 14820–14828. [https://doi.org/10.1007/s11356-](https://doi.org/10.1007/s11356-015-4717-y)
608 015-4717-y

609 Luther, G.W., Rozan, T.F., Witter, a, Lewis, B., 2001. Metal-organic complexation in
610 the marine environment. *Geochem. Trans.* 2, 65. [https://doi.org/10.1186/1467-4866-](https://doi.org/10.1186/1467-4866-2-65)
611 2-65

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

616 Maldonado, M.T., Price, N.M., 1999. Utilization of iron bound to strong organic ligands
617 by plankton communities in the subarctic Pacific Ocean. *Deep. Res. Part II Top.*
618 *Stud. Oceanogr.* 46, 2447–2473.

619 Martin, J.H., Gordon, M., Fitzwater, S.E., 1991. The case for iron. *Limnol. Oceanogr.* 36,
620 1793–1802. <https://doi.org/10.4319/lo.1991.36.8.1793>

621 Millero, F. J., 1986. The pH of estuarine waters. *Limnol. Oceanogr.* 31(4), 839–847.

622 Mira, L., Fernandez, M.T., Santos, M., Rocha, R., Florêncio, M.H., Jennings, K.R., 2002.
623 Interactions of flavonoids with iron and copper ions: A mechanism for their
624 antioxidant activity. *Free Radic. Res.* 36, 1199–1208.

625 <https://doi.org/10.1080/1071576021000016463>

626 Moran, J.F., Klucas, R. V., Grayer, R.J., Abian, J., Becana, M., 1997. Complexes of iron
627 with phenolic compounds from soybean nodules and other legume tissues:
628 Prooxidant and antioxidant properties. *Free Radic. Biol. Med.* 22, 861–870.
629 [https://doi.org/10.1016/S0891-5849\(96\)00426-1](https://doi.org/10.1016/S0891-5849(96)00426-1)

630 Morel, F.M.M., Price, N.M., 2003. The biogeochemical cycles of trace metals in the
631 oceans. *Science*. 300(5621), 944–947. <https://doi.org/10.1126/science.1083545>

632 Morel, F. M., Kustka, A. B., Shaked, Y., 2008. The role of unchelated Fe in the iron
633 nutrition of phytoplankton. *Limnol. Oceanogr.* 53(1), 400-404.

634 Norman, L., Worms, I.A.M., Angles, E., Bowie, A.R., Nichols, C.M., Ninh Pham, A.,
635 Slaveykova, V.I., Townsend, A.T., David Waite, T., Hassler, C.S., 2015. The role
636 of bacterial and algal exopolymeric substances in iron chemistry. *Mar. Chem.* 173,
637 148–161. <https://doi.org/10.1016/j.marchem.2015.03.015>

638 Omanović, D., Garnier, C., Pižeta, I., 2014. ProMCC: An all-in-one tool for trace metal
639 complexation studies. *Mar. Chem.* 173, 25–39.
640 <https://doi.org/10.1016/j.marchem.2014.10.011>

641 Pižeta, I., Sander, S. G., Hudson, R. J. M., Omanović, D., Baars, O., Barbeau, K. A.,
642 Buck, K.N., Bundy, R.M., Carrasco, G., Croot, P.L., Garnier, C., Gerringa, L.J.A.,
643 Gledhill, M., Hirose, K., Kondo, Y., Laglera, L.M., Nuester, J., Rijkenberg, M.J.A.,
644 Takeda, S., Twining, B.S., Wells, M., 2015. Interpretation of complexometric
645 titration data: An intercomparison of methods for estimating models of trace metal
646 complexation by natural organic ligands. *Mar. Chem.* 173, 3–24.

647 Poorvin, L., Sander, S.G., Velasquez, I., Ibisanni, E., LeClerc, G.R., Wilhelm, S.W.,

648 2011. A comparison of Fe bioavailability and binding of a catecholate siderophore
649 with virus-mediated lysates from the marine bacterium *Vibrio alginolyticus* PWH3a.
650 J. Exp. Mar. Bio. Ecol. 399, 43–47. <https://doi.org/10.1016/j.jembe.2011.01.016>

651 Powell, H. K. J., Taylor, M. C., 1982. Interactions of iron (II) and iron (III) with gallic
652 acid and its homologues: a potentiometric and spectrophotometric study. Australian
653 Journal of Chemistry, 35(4), 739–756.

654 Raspor, B., Nürnberg, H.W., Valenta, P., Branica, M., 1980. Kinetics and mechanism of
655 trace metal chelation in sea water. J. Electroanal. Chem. 115, 293–308.
656 [https://doi.org/10.1016/S0022-0728\(80\)80333-0](https://doi.org/10.1016/S0022-0728(80)80333-0)

657 Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999.
658 Antioxidant activity applying an improved ABTS radical cation decolorization
659 assay. Free Radic. Biol. Med. 26, 1231–1237.

660 Rico, M., López, A., Santana-Casiano, J.M., González, A.G., González-Dávila, M., 2013.
661 Variability of the phenolic profile in the diatom *Phaeodactylum tricornutum*
662 growing under copper and iron stress. Limnol. Oceanogr. 58(1), 144–152.
663 <https://doi.org/10.4319/lo.2013.58.1.0144>

664 Rue, E.E.L., Bruland, K.K.W., 1995. Complexation of iron(III) by natural organic ligands
665 in the Central North Pacific as determined by a new competitive ligand
666 equilibration/adsorptive cathodic stripping. Mar. Chem. 50, 117–138.
667 [https://doi.org/10.1016/0304-4203\(95\)00031-L](https://doi.org/10.1016/0304-4203(95)00031-L)

668 Sander, S. G., Hunter, K. A., Harms, H., and Wells, M., 2011. Numerical approach to
669 speciation and estimation of parameters used in modeling trace metal
670 bioavailability. Environ. Sci. Technol. 45(15), 6388–6395.

671 Santana-Casiano, J., González-Dávila, M., González, A.G., Millero, F., 2010. Fe(III)
672 reduction in the presence of catechol in seawater. *Aquat. Geochemistry* 16, 467–482.
673 <https://doi.org/10.1007/s10498-009-9088-x>

674 Santana-Casiano, J.M., González-Dávila, M., González, A.G., Rico, M., López, A.,
675 Martel, A., 2014. Characterization of phenolic exudates from *Phaeodactylum*
676 *tricornutum* and their effects on the chemistry of Fe(II)-Fe(III). *Mar. Chem.* 158,
677 10–16. <https://doi.org/10.1016/j.marchem.2013.11.001>

678 Sato, M., Takeda, S., Furuya, K., 2007. Iron regeneration and organic iron(III)-binding
679 ligand production during in situ zooplankton grazing experiment. *Mar. Chem.* 106,
680 471–488. <https://doi.org/10.1016/j.marchem.2007.05.001>

681 Shaked, Y., Lis, H., 2012. Disassembling iron availability to phytoplankton. *Frontiers in*
682 *Microbiology*, 3, 123.

683 Sroka, Z., Cisowski, W., 2003. Hydrogen peroxide scavenging, antioxidant and anti-
684 radical activity of some phenolic acids. *Food Chem. Toxicol.* 41, 753–758.

685 Stockdale, A., Tipping, E., Lofts, S., Mortimer, R.J.G., 2016. Effect of ocean acidification
686 on organic and inorganic speciation of trace metals. *Environ. Sci. Technol.* 50,
687 1906–1913. <https://doi.org/10.1021/acs.est.5b05624>

688 Strlič, M., Radovič, T., Kolar, J., Pihlar, B., 2002. Anti-and prooxidative properties of
689 gallic acid in fenton-type systems. *Journal of Agricultural and Food*
690 *Chemistry*, 50(22), 6313-6317.

691 Sunda, W., Huntsman, S., 2003. Effect of pH, light, and temperature on Fe–EDTA
692 chelation and Fe hydrolysis in seawater. *Mar. Chem.* 84, 35–47.

693 van den Berg, C.M.G., Kramer, J.R., 1979. Determination of complexing capacities of

694 ligands in natural waters and conditional stability constants of the copper complexes
695 by means of manganese dioxide. Anal. Chim. Acta 106, 113–120.
696 [https://doi.org/10.1016/S0003-2670\(01\)83711-9](https://doi.org/10.1016/S0003-2670(01)83711-9)

697 Vasconcelos, M.T.S.D., Leal, M.F.C., van den Berg, C.M.G., 2002. Influence of the
698 nature of the exudates released by different marine algae on the growth, trace metal
699 uptake and exudation of *Emiliania huxleyi* in natural seawater. Mar. Chem. 77, 187–
700 210. [https://doi.org/10.1016/S0304-4203\(01\)00087-1](https://doi.org/10.1016/S0304-4203(01)00087-1)

701 Witter, A.E., Hutchins, D.A., Butler, A., Luther, G.W., 2000. Determination of
702 conditional stability constants and kinetic constants for strong model Fe-binding
703 ligands in seawater. Mar. Chem. 69, 1–17. [https://doi.org/10.1016/S0304-4203\(99\)00087-0](https://doi.org/10.1016/S0304-4203(99)00087-0)
704

705 Witter, A.E., Luther, G.W., 1998. Variation in Fe-organic complexation with depth in the
706 Northwestern Atlantic Ocean as determined using a kinetic approach. Mar.Chem.
707 62, 241–258. [https://doi.org/10.1016/S0304-4203\(98\)00044-9](https://doi.org/10.1016/S0304-4203(98)00044-9)

708 Wu, J.F., Luther, G.W., 1995. Complexation of Fe(III) by natural organic-ligands in the
709 Northwest Atlantic-Ocean by a competitive ligand equilibration method and kinetic
710 approach. Mar. Chem. 50, 159–177.

711

712 **Acknowledgements**

713 This work was funded by "Laboratoire d'Excellence" LabexMER (ANR-10-LABX-19)
714 and co-funded by a grant from the French Government under the program
715 "Investissements d'Avenir", the Projects EACFe (CTM2014-52342-P) and ATOPFe

716 (CTM2017-83476-P) given by the Ministerio de Economía y Competitividad from Spain
717 and the Postdoctoral program from the Universidad de Las Palmas de Gran Canaria.

Table 1. Formation and dissociation constants, conditional stability constant and half-life time determined using the kinetic method. The initial concentration of dFe = 10.36 nM and the initial concentration of studied ligand was 5 nM.

Studied ligand	$k_f \times 10^5$ ($M^{-1}s^{-1}$)	$k_d \times 10^{-4}$ (s^{-1})	$\log K'_{Fe'L}$	$\log K_{Fe^{3+}L}$	Fe' $t_{1/2}$ (min)	Fe^{3+} $t_{1/2}$ (years)
(±)-Catechin	4.2±1.8	2.43±0.03	9.2±0.1	19.20	20.6	0.54
Sinapic acid	3.2±0.7	4.4±0.3	8.86±0.04	18.86	11.3	0.23
Gallic acid	3.1±0.4	3.2±0.1	9.01±0.02	19.01	15.9	0.32

Table 2. Concentration of studied ligand added and recovered after a titration, conditional stability constant determined using DP-CSV and calculated applying the ProMCC code. Errors represent the standard deviation from three replicates.

Studied ligand	[L]_{added} (nM)	[L]_{recovered} (nM)	$K'_{FeL} \times 10^9$	$\log K'_{FeL}$	$\text{Log} K'_{Fe^{3+}L}$
(±)-Catechin	5.7	1.6±0.2	2.0	9.3±0.1	19.3
(±)-Catechin	11.8	3.6±0.5	1.3	9.1±0.2	19.1
Sinapic acid	5.0	3.6±0.8	0.4	8.6±0.2	18.6
Sinapic acid	9.9	6.5±0.7	0.4	8.6±0.1	18.6
Gallic acid	2.0	5.5±0.3	0.6	8.8±0.2	18.8
Gallic acid	5.0	14±2	3.2	9.5±0.1	19.5

LIST OF FIGURES

Figure 1. Chemical structure of (\pm)-catechin (A), sinapic acid (B) and gallic acid (C)

Figure 2. Example of titration of natural seawater (dots) and 4 hours UV-irradiated seawater (squares).

Figure 3. Kinetic of formation of Fe complexes with (\pm) - catechin (A), sinapic acid (B) and gallic acid (C), where the concentration of dFe is expressed as labile Fe because of $\text{Fe}(\text{TAC})_2$ complex was measured after 20 sec. Kinetic of dissociation of (\pm) - catechin (D), sinapic acid (E) and gallic acid (F), where the concentration of dFe is expressed as TAC labile Fe due to the interaction between TAC and the studied ligands. Dashed lines correspond to the fitting of the results. All the experiments were carried out in triplicate in UV-irradiated seawater. The initial concentration of dFe was 10.36 nM. X-axis are in different scale for each polyphenol due to their reaction time.

Figure 4. Percentage of Fe(II) reduced from Fe(III) in UV-seawater at pH 8.0 and $T=25^\circ\text{C}$ in the presence of (\pm) – catechin, sinapic acid and gallic acid.

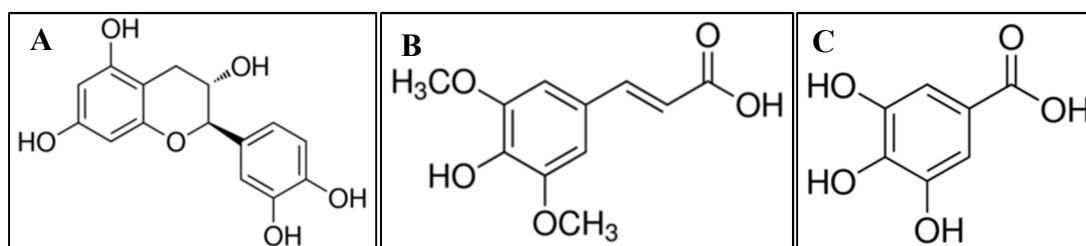


Figure 1. Chemical structure of (±)-catechin (A), sinapic acid (B) and gallic acid (C).

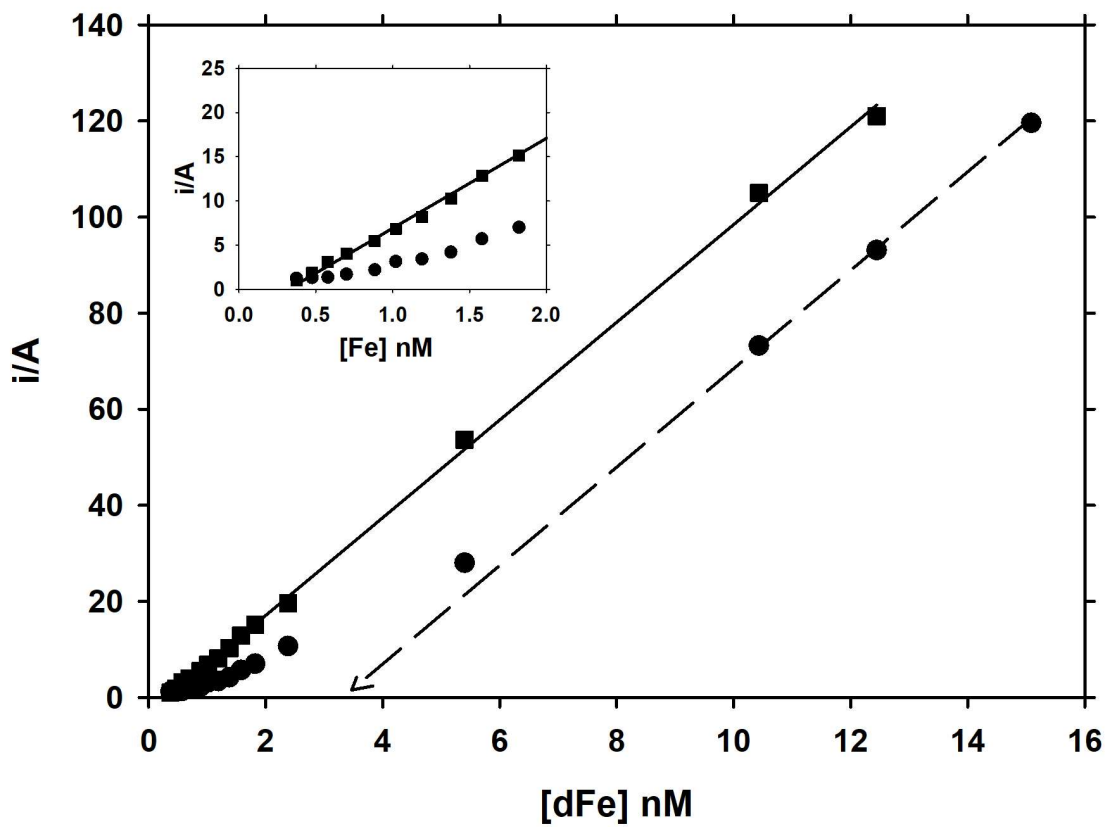


Figure 2. Example of titration of natural seawater (dots) and 4 hours UV-irradiated seawater (squares).

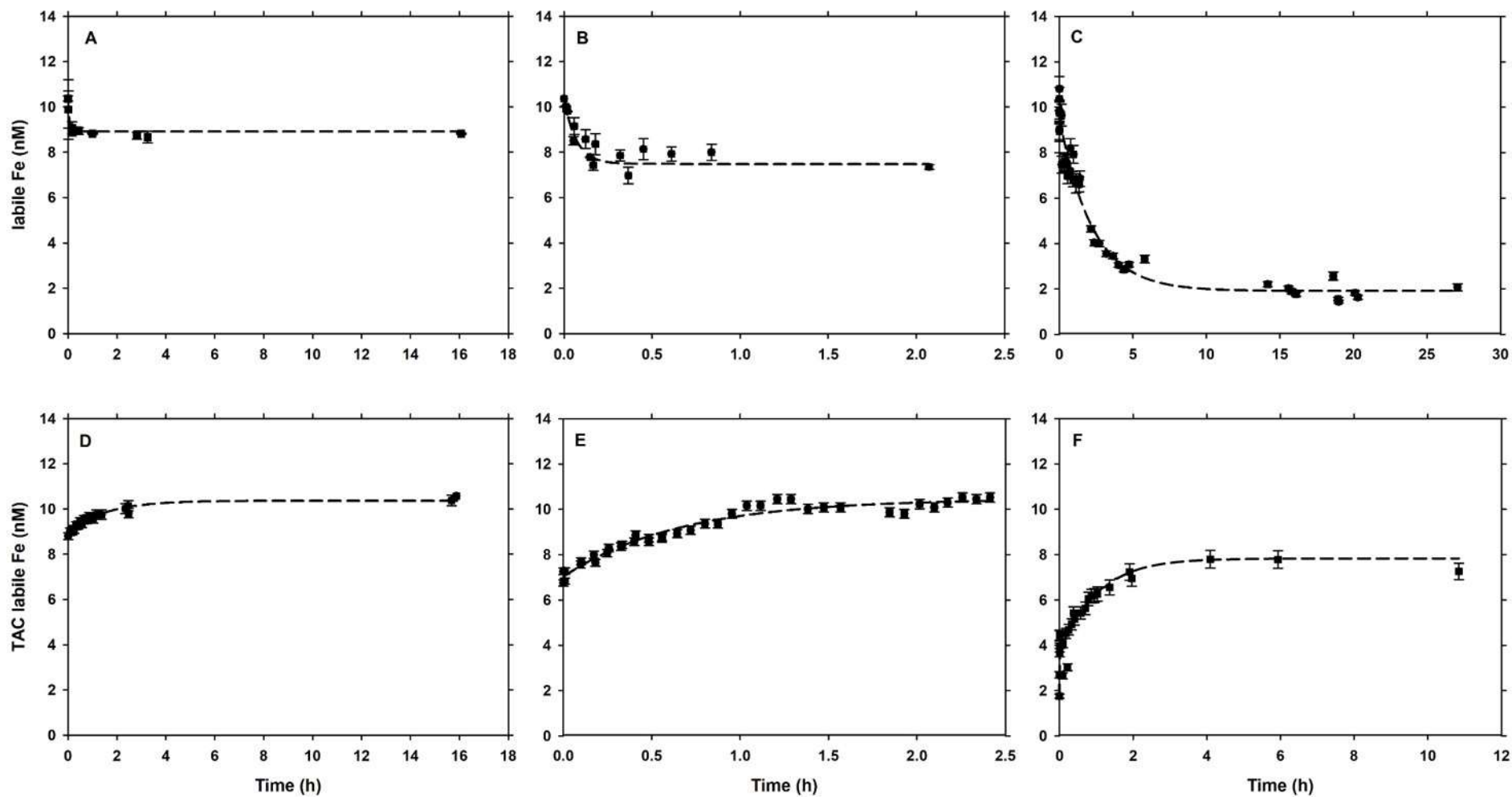


Figure 3. Kinetic of formation of Fe complexes with (±) - catechin (A), sinapic acid (B) and gallic acid (C), where the concentration of dFe is expressed as labile Fe because of Fe(TAC)₂ complex was measured after 20 sec. Kinetic of dissociation of (±) - catechin (D), sinapic acid (E)

and gallic acid (F), where the concentration of dFe is expressed as TAC labile Fe due to the interaction between TAC and the studied ligands. Dashed lines correspond to the fitting of the results. All the experiments were carried out in triplicate in UV-irradiated seawater. The initial concentration of dFe was 10.36 nM. X-axis are in different scale for each polyphenol due to their reaction time.

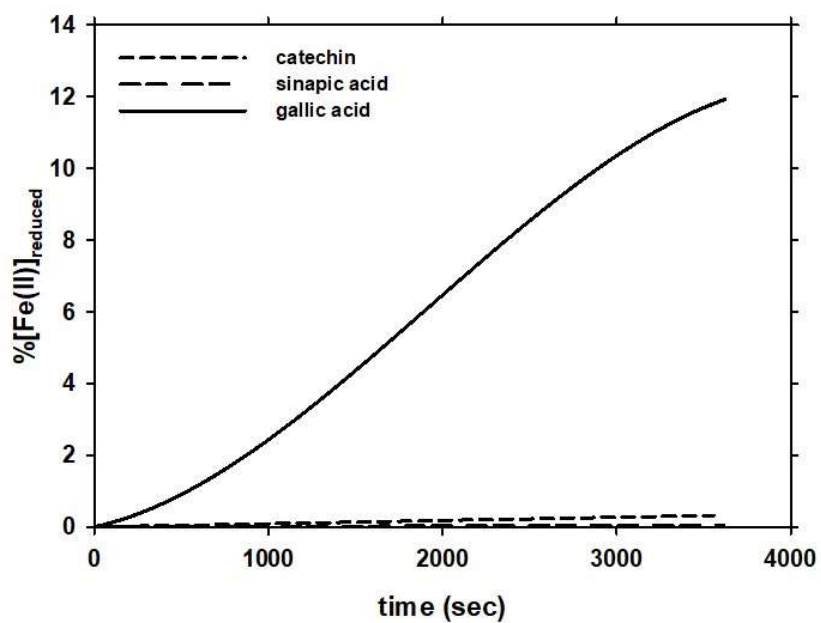


Figure 4. Percentage of Fe(II) reduced from Fe(III) in UV-seawater at pH 8.0 and T=25°C in the presence of (±) – catechin, sinapic acid and gallic acid.