Acid-base properties of dissolved organic matter extracted from the marine environment

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HIGHLIGHTS

• Marine dissolved organic matter affects trace metals biogeochemistry and carbon cycle.
• Research the acid-base properties of DOM is key to unravel its role in marine systems.
• Potentiometric titrations allow us studying the proton binding to marine DOM.
• Marine DOM is compared with fulvic acids in terms of NICA-Donnan parameters.
• Variation in proton binding parameters has potential effects for Fe binding by DOM.

GRAPHICAL ABSTRACT

ABSTRACT

Marine dissolved organic matter (DOM) plays a key role in the current and future global carbon cycle, which supports life on Earth. Trace metals such as iron, an essential micronutrient, compete with protons and major ions for the binding to DOM. These competitive effects and the DOM binding capacity are related to the DOM acid-base properties, which also influence DOM transport and reactivity in marine waters. Here we present the results of a complete set of acid-base titration experiments of a pre-concentrated marine DOM sample in the range 0.01 ≤ I ≤ 0.7 M and 3 ≤ pH ≤ 10. We characterize the obtained proton binding curves using a combination of the non-ideal competitive adsorption (NICA) isotherm and Donnan electrostatic model. Within the main chemical groups of marine DOM, the carboxylic distribution was accurately characterized from the obtained data (Qmax,H1 = 2.52 mol·kg⁻¹, logK(H)1 = 3.26, m1 = 0.69 and b = 0.70). This carboxylic mode was found to be less acidic and more homogeneous than a generic fulvic acid, but the differences are consistent with the reported variability of fulvic acids of freshwater and terrestrial origin. We find that changes in temperature (down to 5.5 °C), and the presence of calcium or magnesium (at 0.01 M) resulted in no significant modification of the proton binding curves obtained at 25 °C and 0.7 or 0.1 M ionic strength, respectively. We demonstrate the relevance of proton binding parameters for the modelling of the system iron/marine DOM throughout a wide range of salinity and acidity conditions in the context of different future ocean scenarios.

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1. Introduction

Dissolved organic matter (DOM) forms one of the largest bioactive carbon reservoirs on Earth and plays a key role in the global carbon cycle (Carvalhais et al., 2014; Hansell, 2013). Marine DOM supports heterotrophic activity and forms complexes with (micronutrient) trace metals, affecting their availability and thus primary production and toxicity. Therefore, marine DOM has major relevance for biogeochemical and climate systems.

Marine DOM is formed of structurally complex and poorly defined compounds (Zark and Dittmar, 2018). The analysis of chemical groups at a molecular level only accounts for ~10% of the DOM pool to date (Repeta, 2015). Carbohydrates, polysaccharides and proteins have been identified as major chemical components of marine labile and semi-labile DOM within the upper water column (Repeta, 2015).

Whilst marine DOM is inherently heterogeneous, carboxylic acids are nevertheless one of the main chemical functionalities in its structure followed in a smaller proportion by phenolic compounds (Hawkes et al., 2019; Hertkorn et al., 2006). Thus, marine DOM possesses fundamental acid-base properties, although investigation of these properties has received little attention to date (Cai et al., 1998; Huizenga and Kester, 1979; Powers et al., 2019; Ulfsbo et al., 2015).

The acid-base behaviour of marine DOM can influence its interactions with particles, including marine microbes, and the description of organic alkalinity of seawater, affecting CO2 system calculations and thus biogeochemical models of the carbon cycle (Kulfiński et al., 2014).

Moreover, the binding of trace metals to marine DOM is controlled by the acid-base properties of DOM and the characteristics of the various chemical species of the metals in seawater, which both depend on pH, ionic strength, temperature, and solution composition (Tipping, 2002). All these factors act in combination impacting biological activity, which is also linked to marine DOM cycling. For example, dissolved iron is bound in ~99% to organic ligands. Since iron limits productivity in as much as 30% of the surface ocean, the overall impact of organic complexation of iron is to increase the dissolved and thus bioavailable iron pool (Gledhill and Buck, 2012). Both iron hydrolysis and iron binding to DOM are strongly influenced by pH, yet a thorough parameterisation of these interactions is currently lacking (Avendano et al., 2016; Gledhill and van den Berg, 1994; Turner et al., 2016).

Potentiometric titrations using glass/selective electrodes are routinely applied for the physicochemical analysis of e.g. biomass (Lodeiro et al., 2019) and fulvic and humic substances of terrestrial or freshwater origin (David et al., 2010; Koopal et al., 2005). In coastal waters like the Baltic Sea, a significant part of the DOM pool is of terrestrial origin and supplied by rivers (Deutsch et al., 2012; Hoikkala et al., 2015; Seidel et al., 2017), although overall a very small fraction of terrestrial DOM (~0.03%) is exported to the ocean (Cai et al., 1998; Carlson and Hansell, 1994; Dittmar and Stubbins, 2014).

The direct physicochemical analysis of marine DOM has proved difficult due to its low concentration, especially in open-ocean environments (< 1 mg/L) (Martín Hernández-Ayon et al., 2007; Yang et al., 2015). Improvements in pre-concentration and isolation techniques such as solid-phase extraction (SPE) have now provided us with powerful tools for marine DOM chemical characterization (Repeta, 2015). Solid-phase extraction mostly isolates hydrophobic fractions of marine DOM and some polar compounds based on well established protocols (Dittmar et al., 2008), with extraction efficiencies >30% for DOC recovery. Pre-concentration techniques selectively restrict the fraction of DOM that is being analysed, which limits our understanding of the full DOM composition. Nevertheless, a careful selection of extraction conditions (flow rate, loading masses and pH) can provide us with DOM extracts that are highly representative for the original seawater sample (Li et al., 2016; Li et al., 2017).

Like marine DOM, terrestrial and aquatic humic-like substances are composed of a large range of different complex molecules. Despite this complexity, generic binding parameters have been obtained, included in speciation software, and used for modelling purposes (Milne et al., 2001; Rey-Castro et al., 2009). We postulate that a similar approach can be implemented for marine DOM in order to allow for the examination of competitive interactions between cations and protons in seawater, and thus a fuller understanding of the impact of ocean acidification on trace metal biogeochemistry in the ocean.

We present here a complete physicochemical study of the acid-base properties of marine DOM isolated from surface Baltic seawater using solid-phase extraction. We applied a simple potentiometric technique for identification of major classes of active chemical groups and measurement of proton binding properties of marine DOM. We used a combination of the non-ideal competitive adsorption (NICA) isotherm that accounts for the specific -chemical- ion binding, and the Donnan model for electrostatic effects (Kinniburgh et al., 1999; Koopal et al., 1994).

Furthermore, we investigated the potential competitive binding effects with two major divalent cations in seawater, i.e.: magnesium and calcium, and the influence of temperature on proton binding. Finally, we address the potential impact of pH and salinity changes on the protonation state of the main chemical groups present in marine DOM with relevance for the binding of iron, and other trace metals.

2. Material and methods

2.1. Seawater collection

Seawater samples were collected in June and July 2019 in a Baltic fjord located in northwest Germany (54.33°N 10.15°E). Surface water (ca. 0.2 m depth) was collected using 20 L acid cleaned high density polyethylene (HDPE) and fluorinated HDPE carboys. A total of approximately 200 L of water were filtered through 0.45/0.2 µm cellulose acetate membrane filters (Sartobran 300, Sartorius) and subsequently acidified with concentrated HCl (Romil UHP grade) to a final concentration of 0.1% (v/v). In order to check for potential organic contamination from the clean carboys used for sampling, three of them were filled with high-purity water with a resistivity of 18.2 MΩ cm⁻¹, and stored overnight. Dissolved organic carbon (DOC) was subsequently measured in triplicate for each of the three carboys (carboy carbon blank).

2.2. Dissolved organic matter extraction

Dissolved organic matter was extracted from the filtered/acidified seawater using Mega Bond-Elut PPL (5 g, 60 mL) solid-phase extraction cartridges (Agilent). The 10 PPL cartridges used were soaked for 12 h with 50 mL of methanol (Fisher Scientific LC-MS grade), and then washed passing 15 mL of HCl 0.1% (v/v) through each cartridge before use. After passing the seawater through, the PPL cartridges were washed with 15 mL of high-purity water, and then soaked during 10 min with (2 x) 10 mL of acetonitrile to finally extract the DOM. The acetonitrile-DOM solution (20 mL from each cartridge) was collected in a Teflon™ pot (250 mL) and dried under a stream of ultrapure N₂ gas for ca. 24 h. The extraction efficiencies were determined as DOC content of the original water sample versus DOC in the extracted seawater after passing 9–12 L through each PPL cartridge. Blanks were obtained in triplicate by extracting high-purity water following the same procedure as for the seawater samples, and determining the DOC concentration in the extracts.

2.3. Dissolved organic matter stock solution

The extracted solid sample (0.74 g) was dissolved in 100 mL of NaOH 0.02 M, and preserved in the dark at 4 °C. This DOM stock solution was diluted and used for the titration experiments.
2.4. Seawater analysis

A detailed description of the seawater analysis for nutrients (phosphate, silicate, nitrate and nitrite), dissolved organic/inorganic carbon, pH, total alkalinity, temperature and salinity, can be found in the Supplementary Material.

2.5. Potentiometric titrations

An automatic titration system controlled with a homemade Matlab script was used for the titration experiments. The system consisted of a burette (Metrohm, Dosimat 765) with a capacity of 1 mL, and a bench-top pH meter (Thermo Orion, 720Aplus) both connected to a computer. The electromotive force between a glass electrode (Metrohm 6.0133.100) and a reference electrode (Metrohm 6.0726.100), with a NaCl jacket at a concentration that matches the ionic strength of the titrated solution, was monitored and recorded using a drift criterion of approximately 0.05 mV/min, with a maximum stabilization time of 120 min. The electrodes were calibrated before each DOM titration in terms of hydrogen ion concentration (Brandariz et al., 2004) using the same ionic strength and potential drift criterion as for the corresponding experiment. Duplicate titrations were performed under water-saturated nitrogen bubbling in a sealed thermostated vessel containing 20 mL of a diluted DOM solution. This solution was made of 10 mL of DOM stock plus different amounts of 2 M NaCl (Merck, p.a.) to set the ionic strength and standardised 0.1 M HCl (Honeywell, Fluka™) to obtain a solution pH 3.0, and diluted with high-purity water. The DOM concentration in all solutions titrated was 1.48 ± 0.07 g DOM·L⁻¹ (436 mg C·L⁻¹). The ionic strength of the titrated solutions was fixed to values of 0.01, 0.02, 0.1 and 0.7 M using NaCl as inert supporting electrolyte. The temperature was kept at 25 °C using a controlled temperature bath circulating water. All experiments were performed under nitrogen saturated conditions and potential drift criterion as for the corresponding experiment. Duplicate titrations were performed under water-saturated nitrogen bubbling in a sealed thermostated vessel containing 20 mL of a diluted DOM solution. This solution was made of 10 mL of DOM stock plus different amounts of 2 M NaCl (Merck, p.a.) to set the ionic strength and standardised 0.1 M HCl (Honeywell, Fluka™) to obtain a solution pH 3.0, and diluted with high-purity water. The DOM concentration in all solutions titrated was 1.48 ± 0.07 g DOM·L⁻¹ (436 mg C·L⁻¹). The ionic strength of the titrated solutions was fixed to values of 0.01, 0.02, 0.1 and 0.7 M using NaCl as inert supporting electrolyte. The temperature was kept at 25 °C using a controlled temperature bath circulator (Thermo Scientific, A10). For the highest ionic strength (0.7 M) experiments at 5.5 and 15 °C were also performed. Standardised 0.1 M NaOH (Honeywell, Fluka™) was added using the burette at 0.025 mL intervals to perform the titration experiments. A typical DOM titration experiment took about 6–8 h.

3. Theory and calculations

3.1. The NICA-Donnan model

The proton titration data was described by the combination of a bi-modal NICA isotherm and the Donnan electrostatic model (Kinniburgh et al., 1996; Kinniburgh et al., 1999; Koopal et al., 1994; Rey-Castro et al., 2009).

In the absence of metal cations able to compete with protons for the specific binding to the functional groups of DOM (mono-component system), the bi-modal NICA isotherm for proton binding is formally identical to the weighted sum of two Langmuir-Freundlich isotherms (Milne et al., 2001):

\[ Q_H = Q_{\text{max}1.1} \left( \frac{(\bar{k}_{1.1} \cdot G_{1.1})^{m_1}}{1 + (\bar{k}_{1.1} \cdot G_{1.1})^{m_1}} \right) + Q_{\text{max}2.1} \left( \frac{(\bar{k}_{1.2} \cdot G_{1.2})^{m_2}}{1 + (\bar{k}_{1.2} \cdot G_{1.2})^{m_2}} \right) \] (1)

where \( Q_H \) stands for the amount of bound protons (mol·kg⁻¹), \( Q_{\text{max}1.1} \) is the total amount of available proton binding sites within each distribution, \( \bar{k}_{1.1} \) is the median value of the \( \rho \)th affinity distribution for protons, \( G_{1.1} \) is the proton concentration at the Donnan phase, and \( m_1 \) (0 ≤ \( m_1 \) ≤ 1) is a parameter related to the width of the affinity distribution function (a measure of the apparent binding heterogeneity). The limiting value of \( m_1 = 1 \) corresponds to a perfectly homogeneous set of sites. The subindexes \( j = 1 \) and \( 2 \) represent the most and less acidic modes in the affinity distribution of sites, usually associated to carboxylic and phenolic functional groups, respectively.

In the presence of bound Ca or Mg (major cations in seawater) or trace metal ions, the system is multi-component and the corresponding competitive NICA expressions are detailed in the Supplementary Material.

The electrostatic contribution to the effective ion binding is accounted for by the Donnan model, which assumes that DOM behaves as an electroneutral, permeable gel phase with a homogeneous distribution of fixed charges (resulting from the dissociation of proton binding functional groups) (Benedetti et al., 1996a; Kinniburgh et al., 1996). The value of the gel phase volume in the Donnan model, \( V_D \), is critical for the ability of the model to accurately reproduce the influence of ionic strength on the shapes of the titration curves. Despite its empirical nature, it is expected to reproduce the influence of the environmental variables such as pH or charge on the hydrodynamic size of the macromolecule. In the absence of experimental estimates, \( V_D \) has been used as a variable to fit the obtained charge curves. The usual fit convergence criterion is the merging of these curves, obtained at different ionic strengths, when they are plotted against the local pH value in the Donnan phase, \( pH_D \), defined as \( - \log C_{\text{H} \cdot \text{D}} \) (termed the ‘master curve’) (Benedetti et al., 1996b).

Three different models of \( V_D \) are compared in this work: 1) standard (or classical) \( V_D \) model; 2) \( V_D \) consistent with Poisson-Boltzmann (PB-\( V_D \)); 3) Ad hoc \( V_D \) values.

The equations and descriptions of the Donnan sub-models used in this work are detailed in the Supplementary Material.

3.2. Strategy for the derivation of NICA-Donnan model parameters

The experimental datasets (titration volume, pH) obtained at each ionic strength were converted into \( \{\text{pH}, \text{charge}\} \) curves using a charge balance relationship, as detailed in the Supplementary Material (Eq S7). Note that pH was measured on the hydrogen ion concentration scale, as described in Section 2.5. The optimization of the NICA-Donnan parameters was carried out by non-linear regression using MATLAB (2019) to minimize the root-mean square error (RMSE, in mol·kg⁻¹) in the DOM charge:

\[ \text{RMSE} = \sqrt{\frac{\sum_{i=1}^{N} (q_i - \bar{q}_i)^2}{N - l}} \] (2)

where \( q_i \) is the fitted value, \( N \) the number of data points and \( l \) the number of model parameters.

In principle, all 6 intrinsic binding constants for proton can be fitted simultaneously together with the \( V_D \) model variable. However, the relatively high degree of covariance among parameters often requires a suitable strategy to constrain the convergence within realistic values. In fact, the characterization of the phenolic distribution from our experimental data proved to be rather uncertain due to poor reproducibility and limited pH range. For this reason, the binding parameters of the second mode were constrained as follows:

- Standard \( V_D \) model: the values of \( \bar{k}_{1.2}, m_2 \), and the ratio \( Q_{\text{max}2.1}/Q_{\text{max}1.1} \) were kept fixed to the corresponding values of the generic NICA parameters for FA (Milne et al., 2001), while the remaining four parameters (\( Q_{\text{max}1.1}, \bar{k}_{1.1}, m_1 \) and \( b \)) were optimized.
- PB-\( V_D \) model: the ratio \( Q_{\text{max}2.1}/Q_{\text{max}1.1} \) was constrained to the generic value for FA, and values of \( \bar{k}_{1.2} \) and \( m_2 \) were kept fixed to the apparent (conditional) values estimated at \( l = 0.7 \) M from the generic FA parameters. In this way, the remaining degrees of freedom in the fits were \( Q_{\text{max}1.1}, \bar{k}_{1.1}, m_1 \) and \( a \).
- ‘ad hoc’ \( V_D \) model: the same as for the PB-\( V_D \) model, but in this case also the intrinsic values of \( Q_{\text{max}1.1}, \bar{k}_{1.1}, m_1 \) were directly fitted from the conditional curve at \( l = 0.7 \) M (which is assumed equivalent to the master curve) and, thus, values of \( V_D \) at \( l = 0.01, 0.02 \) and 0.1 M were optimized to fit the corresponding titration curves.
4. Results

4.1. Marine DOM extracted sample

The marine DOM sample used in the titration experiments was preconcentrated from a surface seawater (Kiel Baltic fjord) with salinity 16 (I = 0.32 M), pH 8.36 ± 0.06 and temperature of 16.6 °C. Values of 2087 ± 9 and 1887 ± 10 μmol·kg⁻¹ were obtained for total alkalinity and dissolved inorganic carbon, respectively. The concentrations of macronutrients were 15.79 ± 0.29 (silicic acid), 0.76 ± 0.23 (phosphate) and 0.11 ± 0.01 (nitrate + nitrite) μmol·kg⁻¹. We calculated an average extraction efficiency of 39 ± 6% for DOC recovery. The measured DOC concentration was 296 ± 5 μmol·kg⁻¹ in the seawater sample, and 182 ± 10 mmol C·L⁻¹ in the prepared DOM stock solution (which corresponded to ca. 7.4 g DOM·L⁻¹). Carboy carbon blanks showed DOC values (3.16 ± 5.99 μmol/L) of the same order of magnitude of the blanks (acidified high-purity water) run in DOC analysis. The blanks for the PPL extraction procedure also yielded negligible DOC values (3.42 μmol C) with a contribution of C to the DOM stock solution below 0.1%. Thus, we observed a negligible carbon contribution from the SPE cartridges or filters that we used in our study.

4.2. Acid-base properties of marine DOM

Fig. 1 shows the comparison between experimental data and model fits using the optimized parameter values listed in Table 1. Fig. 2 shows the density of probability of proton binding affinities (i.e., the affinity spectra) of marine DOM sites for two different reference states: a) intrinsic conditions (i.e., chemical binding affinities); and b) effective conditions (chemical plus electrostatic binding) at the sampling site, i.e.: I = 0.32 M. The corresponding spectra calculated with the NICA-Donnan parameters of a generic fulvic acid were included for comparison in both cases. The intrinsic affinity spectra in panel (a) reflect the binding characteristics of the master curves obtained with each of the three Donnan models studied. Panel (b) also shows the density of protonated sites for marine DOM at pH 8.36. The spectra were calculated as described in Rey-Castro et al. (2009) (see Supplementary Material).

4.3. Marine DOM Donnan volumes and radii

Fig. 3 shows the values of V_D obtained with the different Donnan models compared in this work and those obtained from the generic value of b for fulvic acids. Table 2 includes a summary of values (radii and average distance between charges) derived for marine DOM on the basis of the fitted values of V_D.

4.4. Effect of temperature and presence of major ions on proton binding

Table S1 shows the mean affinity of the 1st (carboxylic) distribution obtained from titrations at I = 0.7 M in the range between 5.5 and 25 °C. The experimental data and NICA-Donnan predictions of the titrations carried out in 0.01 M Ca²⁺/Mg²⁺ (at I = 0.1 M) are shown in Fig. S1.

4.5. Relevance to modelling of trace metal binding by marine DOM

Fig. 4 shows theoretical NICA-Donnan predictions of iron binding by marine DOM using two different combinations of proton binding parameters, for a range of salinity (S) and pH conditions representative of surface waters in the Baltic Sea, and assuming inorganic dissolved...
iron controlled by the solubility of Fe(OH)$_3(s)$. These calculations are only intended to illustrate the importance of an exhaustive characterization of acid-base and electrostatic features of marine DOM as a first approximation in the development of predictive models for the effects of climate change scenarios on the availability of trace metals in the ocean.

5. Discussion

5.1. Seawater analysis

The sampled surface brackish water presents typical characteristics of sea areas influenced by both terrestrial freshwater inputs and seawater inputs from the North Sea. The high pH value obtained (8.36) is consistent with surface waters at the end of a phytoplankton bloom, which is confirmed by relatively low nutrient concentrations. Since the water was post-bloom, it is likely there was intense production of autochthonous organic matter, which contributed to high DOC values (296 μmol·kg$^{-1}$). Moreover, we measured relatively low alkalinity and dissolved inorganic carbon concentrations of ca. 2000 μmol·kg$^{-1}$, which is a typical value for this kind of large estuarine systems (Kuliński et al., 2014).

5.2. Acid-base properties of marine DOM

The experimental acid-base titration curves of the extracted marine DOM at 25 °C show the typical shape of a bi-modal Langmuir-Freundlich isotherm. These curves were successfully fitted with the mono-component NICA-Donnan model for proton ions within the

<table>
<thead>
<tr>
<th>Donnan sub-model</th>
<th>$V_D$ parameter</th>
<th>$Q_{\text{max, } 1}$ (mol·kg$^{-1}$)</th>
<th>$m_1$</th>
<th>$Q_{\text{max, } 2}$ (mol·kg$^{-1}$)</th>
<th>$m_2$</th>
<th>RMSE (mol·kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Eq. S12</td>
<td>$b = 0.70$</td>
<td>2.52</td>
<td>3.26</td>
<td>0.69</td>
<td>0.80</td>
<td>8.60$^b$</td>
</tr>
<tr>
<td>Eq. S13</td>
<td>$a = 0.036$</td>
<td>2.55</td>
<td>3.82</td>
<td>0.59</td>
<td>0.81</td>
<td>9.41$^c$</td>
</tr>
<tr>
<td>‘ad hoc’</td>
<td>Reference: $I = 0.7$ M</td>
<td>2.52</td>
<td>3.79</td>
<td>0.55</td>
<td>0.80</td>
<td>9.41$^c$</td>
</tr>
</tbody>
</table>

The values in bold were constrained as follows: * The ratio $Q_{\text{max, } 2}/Q_{\text{max, } 1}$ was kept fixed at 0.32 as in the generic parameterization for fulvic acid (Milne et al., 2001). * Generic values for fulvic acid (Milne et al., 2001). * Estimated at $I = 0.7$ M using the generic intrinsic NICA parameters for fulvic acid. The comparison between NICA-Donnan model and experimental values is shown in Fig. 1.

Fig. 2. (a) Intrinsic proton affinity spectra of marine DOM calculated from the NICA parameters of Table 1 using: standard $V_D$ (blue), PB-$V_D$ (green) and ‘ad hoc’ (black) Donnan models. (b) Effective proton affinity spectrum of marine DOM at $I = 0.32$ M (solid blue line) and density of protonated DOM sites at pH 8.36 (shaded blue area), calculated with NICA and standard $V_D$ parameters from Table 1 (the three Donnan models lead to virtually identical effective spectra). The intrinsic and effective ($I = 0.32$ M) spectra of a generic fulvic acid (Milne et al., 2001) are included as red dashed lines in panels (a) and (b), respectively, for comparison purposes. All spectra are normalized. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Values of $V_D$ obtained from fits to proton titration data of marine DOM at 25 °C using different Donnan models: PB-$V_D$ (squares), ‘ad hoc’ (open circles), standard model using fitted $b$ parameter (blue line), and standard model using the generic value of $b$ for fulvic acids (red dashed line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Donnan model assuming that free Fe\(^{3+}\) ion concentration is controlled by the solubility a function of salinity (S) and pH within the typical values of surface waters of Baltic Sea (this article.)

reference to colour in this cases. See Supplementary Material for further details. (For interpretation of the fulvic acid (Milne et al., 2001). The binding parameters of Fe(III) are identical in both

Table 2

<table>
<thead>
<tr>
<th>I (mol·L(^{-1}))</th>
<th>(\kappa^{-1}) (nm)a</th>
<th>(V_0) (L·kg(^{-1}))b</th>
<th>(\kappa\cdot r^f)</th>
<th>(\kappa\cdot d_{av}^f)</th>
</tr>
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<tbody>
<tr>
<td>0.01</td>
<td>3.6</td>
<td>16–71</td>
<td>0.4–0.7</td>
<td>0.7–1.1</td>
</tr>
<tr>
<td>0.02</td>
<td>2.3</td>
<td>9–49</td>
<td>0.5–0.9</td>
<td>0.9–1.6</td>
</tr>
<tr>
<td>0.1</td>
<td>1.0</td>
<td>2.6–17</td>
<td>0.9–1.6</td>
<td>1.5–2.7</td>
</tr>
<tr>
<td>0.7</td>
<td>0.4</td>
<td>0.7–n.a.</td>
<td>1.7–n.a.</td>
<td>2.8–n.a.</td>
</tr>
</tbody>
</table>

a Debye length at 298 K.
b Range of fitted \(V_0\) values, where lower and upper bounds corresponds to standard and 'ad hoc' Donnan models, respectively (see Fig. 3).
c Values calculated with eq. S15 using \(\rho = 1.4-2.0\) kg/L (Benedetti et al., 1996b) and a reference \(M_w\) value of 500 Da.
d Average distance (in Debye length units) between charged groups calculated as \(d_{av} = 2r/\kappa\cdot Z^f_{\text{max}}\) using an average amount of charged groups per molecule \(Z_{\text{max}} = M_w \times (Q_{\text{max}})^{-1}\) (reference \(M_w = 500\) Da).
e The 'ad hoc' model assumes negligible electrostatic contribution at \(I = 0.7\) M.

Fig. 4. Contour plots of the estimated Fe(III) binding to marine DOM, \(Q_{\text{max}}\) (mmol·kg\(^{-1}\)), as a function of salinity (S) and pH within the typical values of surface waters of Baltic Sea (the red circle represents actual conditions at the sampling site), calculated by NICA-Donnan model assuming that free Fe\(^{3+}\) ion concentration is controlled by the solubility equilibrium of Fe(OH)\(_2\)(OH) at 15 °C. Dataset (a): values of \(Q_{\text{max}}\), \(\kappa\cdot Z^f_{\text{max}}\), \(\kappa\cdot r^f\), \(\kappa\cdot d_{av}^f\), and \(\kappa\cdot d_{av}^f\) reported in this work; dataset (b): generic values of \(Q_{\text{max}}\), \(\kappa\cdot Z^f_{\text{max}}\), \(\kappa\cdot r^f\), \(\kappa\cdot d_{av}^f\), and \(\kappa\cdot d_{av}^f\) for fulvic acid (Milne et al., 2001). The binding parameters of Fe(III) are identical in both cases. See Supplementary Material for further details. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

range 0.01 ≤ I ≤ 0.7 M and 3 ≤ pH ≤ 10 (Fig. 1). The values of RMSE (Table 1) are comparable to those obtained from fits of individual datasets of terrestrial and freshwater fulvic acid samples, and are certainly lower than values (RMSE = 0.123) obtained with the collective dataset used to derive the generic NICA parameters for FA (Milne et al., 2001). Therefore, and from a purely empirical point of view, the combination of NICA and the standard Donnan model proved to be an accurate descriptor of the proton binding behavior of marine DOM.

We were able to accurately characterize and fit the 1st group of binding sites (the low affinity distribution) from the experimental titration data (Table 1). However, for the 2nd group (the high affinity distribution), a direct (unconstrained) fitting yielded excessively wide confidence intervals for \(Z^f_{\text{max,2}}\) and \(m_2\) values. This was a result of) little overlap between both distributions, as shown in Fig. 1, and ii) relatively poor reproducibility of experimental data above pH 9. We therefore decided to fix \(Z^f_{\text{max,2}}/Q_{\text{max,2}}\) ratio, to the generic values of the phenolic groups of FA (Milne et al., 2001), as explained in Section 3.2. This restriction in the degrees of freedom did not increase the value of the RMSE substantially, as compared with the optimization of the full set of NICA parameters (data not shown). This suggests that the generic phenolic parameters of FA might be close to the actual intrinsic values of the 2nd distribution of marine DOM sites. In any case, future work should be carried out within an extended pH interval and a reduced tolerance in the mV/min shift criterion in order to achieve a more robust assessment of the features of the high affinity binding sites.

We observed some differences between our marine DOM and the typical behavior of fulvic acids of terrestrial origin:

1) Number of titratable groups: Our marine DOM has a relatively small amount of groups (2.52 mol·kg\(^{-1}\)) as compared with average (generic) values reported for FA (ca. 6 mol·kg\(^{-1}\)). Here, we would like to highlight the potential bias introduced by the technique used to preconcentrate our marine DOM sample. Solid-phase extraction of marine DOM using PPL columns tends to selectively retain hydrophobic compounds. Moreover, the extraction yield is usually far from being complete, with a 39% recovery based on DOC values in our case. This selective and incomplete DOM extraction procedure could therefore limit the number and type of chemical compounds analysed in the titration experiments. Nevertheless, we selected the experimental conditions based on previous studies that suggest that marine DOM extracted at pH 2 using PPL cartridges is representative of bulk marine DOM (Green et al., 2014; Li et al., 2016; Li et al., 2017). To confirm this, further validation and improvement of the extraction methods with respect to retention of polar components will be an objective for future research. In any case, the
methodology of this work may be extended to the individual characterization of separate DOM fractions collected through a multi-stage sequential extraction scheme (using e.g. adsorption resins of different hydrophobicity). The collective behaviour of the whole DOM in the original seawater sample could, then, be described by the weighted average (relative to the respective number of binding sites) of the spectra corresponding to each individual fraction of DOM (Christl and Kretzschmar, 2001; Pernet-Coudurier et al., 2011).

2) Intrinsic (chemical) proton affinity: The fitted NICA parameters indicate that the low affinity distribution of marine DOM is slightly less acidic and significantly more homogeneous than an average FA, which presents generic values of $\log K_{f1,1} = 2.34$ and $m_1 = 0.38$ (recall that $m = 1$ corresponds to a perfectly homogeneous distribution). The differences in proton binding behaviour between marine DOM and the generic FA can be visually identified in the intrinsic affinity spectra of Fig. 2a. Still, the variability observed in literature in the NICA parameters obtained from individual fits to specific FA samples is substantial. For instance, Milne et al. (2001) report the following min-max ranges: $2.64 < Q_{\text{max, } f1} \leq 8.76$ mol·kg$^{-1}$, $2.00 < \log K_{f1,1} < 3.81$; and $0.27 < m_1 < 0.65$ in an ensemble of 25 datasets. In conclusion, the characteristics of the most acidic mode of the marine DOM fraction are notably different from the carboxylic mode of an average (generic) FA, but the deviations are almost within the observed natural variability between samples.

3) Effective (conditional) proton affinity: The generic NICA-Donnan set of parameters for FA lead to effective binding spectra that are clearly different from those of marine DOM as displayed in Fig. 2b for the particular case of $I = 0.32$ M. At these conditions, the effective affinity spectrum of marine DOM shows a first peak (with a maximum at $\log K_{f1,1} = 3.8$) that is noticeably narrower (i.e., less heterogeneous) and slightly shifted to larger affinities ($0.3 \log$ units) as compared with the generic FA. For the standard $V_D$ model, the fitted value of $b$ (0.70) is above the generic FA value (0.57), indicating that the values of $V_D$ in the marine DOM fraction are somewhat larger (and more sensitive to variations in ionic strength) than in a generic FA. Yet the high variability observed (0.29 $< b < 0.94$) in fits of individual fulvic datasets (Milne et al., 2001) suggests, again, that the electrostatic behaviour of marine DOM may not be inherently different from that of a terrestrial FA. However, one controversial issue remains as seen in Fig. 1, the master curve obtained with the standard Donnan model deviates significantly from the titration curve at $I = 0.7$ M, suggesting that electrostatic effects are still relevant at this high ionic strength, which does not seem plausible. This is the reason why an alternative fit with the other two models was carried out as a comparison.

As regards the PB-$V_D$ and ‘ad hoc’ models, it can be noticed (see Table 1) that the total amount of sites obtained from fitting is almost the same as in the standard model (3.36–3.32 mol·kg$^{-1}$), but the intrinsic (chemical) values of the low affinity distribution now display larger average and variance values (Fig. 2a), i.e.: the 1st group of sites are less acidic and more heterogeneous than in the standard model. It must be noted that the differences among the three different Donnan models lie mainly on how the free energy of proton binding is split into intrinsic (chemical) and electrostatic contributions. Indeed, the effective (overall) binding affinity distributions at a given ionic strength for marine DOM are almost indistinguishable from each other.

When we compare our PPL-extracted DOM with the only study found on acid-base properties of extracted marine DOM (Huizenga and Kester, 1979), we observed certain similarities:

1) The number of titratable groups of the first mode of our DOM (8.56 mmol/g C) is close to the lower range of the values, 8.96–13.82 mmol/g C, shown by Huizenga and Kester (1979) for DOM isolated by activated charcoal adsorption from river, estuarine, coastal, and open ocean waters.

2) The log $k$ values obtained by Huizenga and Kester (1979) in NaCl of 0.7 m for the eight samples of marine DOM studied lie between 3.33 and 3.75, which is in agreement with the value that we calculated for the first mode, 3.79 at 0.7 M NaCl.

These authors present results within the pH range 2–8, and therefore they were not able to characterize the binding sites with affinity distribution associated to phenolic functional groups.

5.3. Marine DOM Donnan volumes and radii

The values of $V_D$ (Fig. 3) show in all cases a decreasing trend with $I$, which is the expected behaviour due to screening of intramolecular electrostatic repulsion. The values obtained with the PB-$V_D$ and ‘ad hoc’ models are very similar and significantly larger than those fitted with the standard model. Consequently, the former two models yield master curves that lie close to the curve at the highest ionic strength, which is a reasonable behavior. However, these relatively large values of $V_D$ may lose some of the underlying theoretical significance. For instance, the PB-$V_D$ sub-model leads to a fitted value of $a = 0.036$ kg·mol$^{-1/2}$·L$^{1/2}$ (Table 1) which corresponds to a solid-sphere radius of 0.1 nm (assuming $\rho = 1.4$ kg·L$^{-1}$). This value is representative of a molecular (rather than nanoscopic) scale. Additionally, the $V_D$ values obtained with standard and ‘ad hoc’ models are translated here into estimated DOM radii on the basis of a reference molecular weight value for marine DOM of 500 Da, in agreement with the values reported in literature (Dittmar and Stubbins, 2014; Hawkes et al., 2019; Seidel et al., 2017). In the standard model, the sizes obtained (Table 2) are just below the characteristic Debye lengths ($\kappa^{-1}$) at the corresponding ionic strengths up to 0.1 M, and the average charge-charge distance becomes close to $\kappa^{-1}$ already at 0.02 M, whereas the ‘ad hoc’ model predicts consistently larger dimensions. Thus, the size and charge distribution of marine DOM (particularly at high ionic strength) might not be fully consistent with the postulates of the Donnan model (Ohshima, 2006; Ohshima, 2008). A more realistic description of the electrostatic effects (probably through an oligoelectrolyte model) seems then to be required, although it lies out of scope of this work. Nevertheless, the use of the NICA-Donnan model is still relevant, as it allows us to compare the behavior of the isolated marine DOM fraction with the generic descriptions of fulvic acids of terrestrial and freshwater origin, in terms of the average and variance of the corresponding proton affinity distributions (Fig. 2).

5.4. Effect of temperature and presence of major ions on proton binding

We observed no difference in the titration curves obtained at three different temperatures beyond experimental uncertainty. Still, we could estimate an upper bound for the absolute value of the average protonation enthalpy of the carboxylic sites as $24$ kJ·mol$^{-1}$ (see Supplementary Material), which is consistent with previous literature reports (Xu et al., 2018).

The effects of calcium and magnesium ions on the proton binding to marine DOM were also investigated, with inconclusive results. Acid-base titrations in presence of 0.22 M Ca$^{2+}$ (at $I = 0.7$ M) led to physically unreasonable results, and a gelatinous precipitate was observed at the end of the experiments. On the other hand, duplicate titrations carried out in 0.01 M Ca$^{2+}$ or Mg$^{2+}$ (at $I = 0.1$ M) yielded no apparent precipitate and almost identical results as in the absence of calcium/magnesium at the same ionic strength. The titration curves were compared with NICA-Donnan predictions using the fitted parameters from Table 1 and the generic values for the intrinsic binding parameters of
Ca^{2+} and Mg^{2+} (Milne et al., 2003), since the experimental information is not enough to perform an optimization of the latter parameters. The results (see Supplementary Material) are consistent with negligible calcium/magnesium binding and, consequently, almost no competition between protons and calcium or magnesium ions under these conditions.

5.5. Relevance to modelling of trace metal binding by marine DOM

The NICA-Donnan model assumes that trace metal ions compete to a greater or lesser degree with protons and major ions for the binding to macromolecular sites. The extent of this is the result of a complex interplay among the concentration of competing ions, their intrinsic affinity parameters, ionic strength and inorganic speciation of the metal ions, e.g. hydrolysis (Rey-Castro et al., 2009). To illustrate this, we take the example of Fe(III), a bioessential micronutrient. We compare the NICA-Donnan predictions of the amount of iron bound by marine DOM ($Q_{Fe}$) as a function of salinity and pH using two different sets of parameters (see Fig. 4). These sets differ exclusively on the proton binding parameters, while the values for Fe(III) are the same in both cases and taken from a previous work (Avendaño et al., 2016). The contour plot in panel (a) was calculated with the marine DOM values fitted in this work (parameter set a); whereas panel (b) used the corresponding generic values for fulvic acid (parameter set b) (Milne et al., 2001). Further details are given in the Supplementary Material.

As can be observed, the parameter set (a) leads to values of $Q_{Fe}$ that are ca. one order of magnitude lower than parameter set (b) at the same environmental conditions.

The conditional affinity spectra for Fe(III) in the calculated scenarios (see Supplementary Material) show that the competitive effect of protons (and, to a much lesser extent, Ca and Mg) induces a shift in the effective affinity of phenolic sites for iron which results in an overlap with the carboxylic mode (which remains mostly unoccupied, as shown in Fig. 2b). This means that both kinds of sites participate in the binding of iron ions. Consequently, the differences observed in the amount of iron bound (Fig. 4) are due to:

1) The decreased degree of heterogeneity of the first mode of binding sites in the extracted marine DOM. There is therefore a smaller fraction of carboxylic sites in the marine DOM with a high affinity for Fe(III) ions.
2) The variation in the maximum binding capacities: $Q_{max, \text{I}} + Q_{max, \text{II}}$ of marine DOM is ca. 60% lower than in the generic FA.
3) The differences in the Boltzmann factor for both marine DOM and generic FA. The values of $V_D$ are somewhat larger for DOM than generic FA at the same salinity. The ion concentrations in the Donnan phase are therefore lower at the same ionic strength and macromolecular charge and, thus, the variation of $Q_{Fe}$ with salinity is less pronounced for marine DOM (Fig. 4, panel (a)).

Finally, the relative gradient of $Q_{Fe}$ with pH at constant salinity is similar for both marine DOM and generic FA, as it is mainly controlled by the dependence of iron hydroxide solubility with pH, while the H/Fe(III) exchange ratios are quite similar in both datasets. A more extensive discussion is included in the Supplementary Material.

We must acknowledge the limitations in the predictive capacity of these scenarios, since the values of the iron binding parameters have not been optimized for this specific DOM fraction. Still, the differences between both scenarios reflect the potential implications of the variation in electrostatic and chemical proton binding parameters with the composition of DOM. In this way, the results of this work represent a useful first step in the development of a systematic model for the prediction of the ion binding characteristics of marine DOM. In addition to optimisation of extraction procedures and improvement in consistency in titration data obtained at pH > 9.5, future work should also address the correlation of the model parameters with spatial and seasonal variability of extracted DOM to achieve a representative description.

5.6. Broader environmental implications

Climate change effects will produce variations in pH, temperature, and oxygen concentrations, with associated effects, in seawaters. Alterations in the chemical composition of marine DOM due to global shifts in the future ocean are expected. This broad pool of organic molecules contribute to the storage of atmospheric CO$_2$ in the ocean, support ecosystems and influence (micronutrient) trace metal cycles, which are essential for marine primary production, but can also produce toxic effects. For example, these organic ligands are thought to increase iron solubility and thus the overall iron inventory in the ocean, since via competition with hydroxide ions, they counteract the tendency for precipitation of iron hydroxides (Liu and Millero, 2002). Marine DOM-metal interactions are key to our understanding of trace metal binding behaviour in seawater and should therefore be considered in global biogeochemical models to evaluate future climate change scenarios, such as ocean acidification (Avendaño et al., 2016; Gledhill et al., 2015; Ye et al., 2020). All these complex interactions depend on the physicochemical properties of the groups that form the marine DOM. Determination of the acid-base properties of DOM, which we investigate here, can provide an accurate and quantitative description of these chemical groups.

6. Conclusions

The NICA-Donnan model was able to fully describe the most acidic mode (carboxylic groups) of marine DOM. This mode has a larger mean affinity for protons and a lower heterogeneity degree than a generic fulvic acid but still within the reported variability for fulvic acids of freshwater and terrestrial origin. Our DOM binding parameters are similar to the few values found in literature for DOM extracted from estuarine, coastal, and open ocean waters. No apparent effect of temperature (between 5 and 25 °C) or major seawater cations (Ca/Mg at representative concentrations) was noticed in the proton binding curves of marine DOM. The binding parameters derived from acid-base titrations are relevant for the prediction of binding of a trace metal such as iron to marine DOM.

Data statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Pablo Lodeiro: Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Supervision. Carlos Rey-Castro: Conceptualization, Methodology, Writing - original draft, Writing - review & editing. Calin David: Methodology, Software, Validation, Formal analysis, Data curation, Visualization. Eric P. Achatberg: Resources, Writing - review & editing. Jaume Puy: Resources, Writing - review & editing. Martha Gledhill: Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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Appendix A. Supplementary data

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References


